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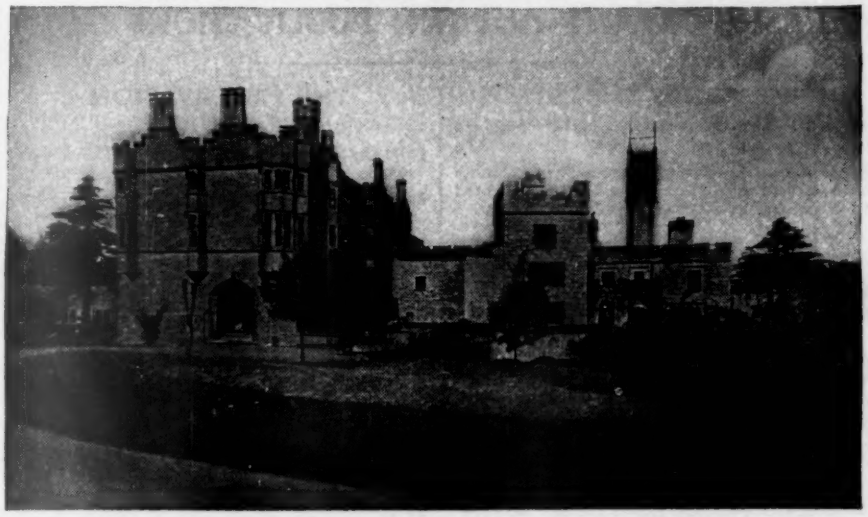

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By ANDREAS FANCONI and G. ALAN ROSE

(From the Medical Unit, University College Hospital, London)

The study of calcium metabolism in health and disease has been greatly impeded by the difficulty of determining the distribution of calcium in the plasma between the three main forms in which it may occur. These are the ionized, protein-bound, and complexed forms. In the present paper the term 'complexed' refers to that fraction of calcium which is ultrafilterable but not ionized. Most of this calcium is complexed to small diffusible molecules, the chief such molecule being citrate. The only accepted method of determining the ionized calcium of plasma has been the biological method of McLean and Hastings (1934), in which they used the frog heart as an indicator for calcium ions. This method has been applied by Compere, McLean, and Hastings (1935) to the study of rickets, and by Sartori (1955*a*) to the study of ultrafiltrates of serum from patients with rickets and hyperparathyroidism and also from infants with hypercalcaemia (1955*b*). Unfortunately the frog-heart technique suffers from many of the usual hazards of a biological method, and may be unsuitable for determinations in plasma from patients with pathological conditions. In these circumstances unknown substances may be present in the plasma, or known substances may be present in abnormal concentration without such departure from normal being suspected. In particular, magnesium ions or organic acids may be present in abnormal concentration, and may affect the frog heart. A purely chemical method which permits the determination of ionized and ultrafilterable calcium in human plasma has recently been described by Rose (1957). It is not susceptible to the hazards of the biological method, and has now been applied to the plasma of patients with various disturbances of calcium metabolism. Attempts have been made, first to verify that the method is valid in pathological conditions, especially those affecting acid-base balance; secondly, to see what further theoretical information could be obtained from measuring ionized and ultrafilterable calcium, rather than total plasma calcium alone; and thirdly, to see whether determinations of plasma ionized calcium might be an aid to diagnosis. It was especially fortunate that it was possible to select

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for study the patients from the Metabolic Ward of University College Hospital, where many rare disorders of calcium and phosphorus metabolism were being extensively investigated and treated under Professor C. E. Dent, as well as from the general medical wards of the Hospital. A survey has been carried out in which the plasma calcium fractions were determined in patients with a number of conditions affecting calcium metabolism. The results of this survey are reported in the present paper. Some of them are unexpected, and in some instances deductions made from them have been based upon relatively small numbers of observations. Nevertheless, such results and deductions are presented, since, while it will be impossible for the present authors to investigate further many of the points in question, others may be stimulated to do so.

Methods

The plasma calcium fractions were determined by the method of Rose (1957), with the following modifications:

1. All blood was taken from patients in the fasting state at about 9 a.m., except that a single cup of tea was allowed two to three hours beforehand.

2. A sample of alveolar air was collected within a few minutes of the venepuncture. The carbon-dioxide content was determined in a standard Haldane gas-analysis apparatus. A synthetic 'alveolar air' of the same carbon-dioxide tension was then made as required, by mixing air and 5 per cent. (or 10 per cent.) carbon dioxide in oxygen, and moistening the mixture with normal saline. Each gas passed through a flow-meter fitted with a tap which allowed the rate of flow to be regulated so as to give a mixture of the desired composition. This gas mixture was then used in lieu of the 5 per cent. carbon dioxide used for normal subjects by Rose (1957). None of the subjects investigated had pulmonary disease.

3. Total plasma calcium, as well as total ultrafilterable calcium, was determined by the method of Fales (1953) as modified by Kenny and Toverud (1954). This method is an ethylenediamine-tetra-acetic acid (EDTA) titration with murexide indicator.

4. It was sometimes found, especially in warm weather, that if the determination of ionized calcium was delayed for an hour or two the ultrafiltrate might become turbid because of bacterial growth. It was then necessary to centrifuge the ultrafiltrate immediately before making the determination. The turbidity was not due to loss of carbon dioxide and precipitation of calcium carbonate, since the ultrafiltrate was equilibrated with carbon-dioxide mixture before centrifuging. Furthermore, if an ultrafiltrate was allowed to become turbid and then cleared by the centrifuge, the extinction observed with the spectrophotometer was restored to the initial level. The difficulty could be completely avoided by the addition of a trace of pure chloramphenicol to the ultrafiltrate, and this has now become our standard practice.

Plasma bicarbonate and potassium, and urine calcium, were determined in

Case number	Patient	Age (years)	Sex	Main diagnosis	Plasma calcium fractions: Total in plasma	In ultrafiltrate (mg./100 ml.): Total	Ionized	Complexed	In plasma water: Total (mg./100 g.)	Ionized (mg./100 ml.)	Protein-bound (mg./100 ml.)	Plasma proteins* (g./100 ml.)	Alveolar CO_2 (mm. Hg)	pH (Calculated)	Various (Urine Ca mg./24 hrs.; Blood urea mg./100 ml.)	Plasma: Phosphorus† (mg./100 ml.)	Bicarbonate (m-equiv./l.)	Remarks‡	Case number
1	D. H.	23	M	Normal	9·17	5·93	5·6	0·33	9·78	5·88	3·57	6·8	34·8	..	..	..	..	.. ..	1
				overbreathing	10·58	5·62	4·5	1·12	11·29	4·73	5·44	7·24	14·2	..	..	..	..		
2	M. L.	32	M	Normal	10·0	6·38	6·2	0·18	10·62	6·51	3·93	6·64 (4·36)	36·8	7·47	..	..	27·1	.. ..	2
				overbreathing	10·15	5·7	4·5	1·2	10·79	4·73	4·86	..	13·9	7·82	..	..	22·4		
3	M. W.	34	M	Normal	9·3	6·1	5·7	0·4	9·93	5·99	3·54	7·2	37·1	7·42	..	..	24·4	.. ..	3
4	B. R.	24	M	Normal	9·18	6·07	5·66	0·41	9·79	5·95	3·43	6·88 (4·29)	35·7	7·43	..	..	23·8	.. ..	4
5	J. S.	26	M	Normal	9·65	6·35	6·18	0·17	10·31	6·48	3·66	7·23 (4·88)	37·0	7·46	..	..	26·3	.. ..	5
6	G. C.	24	M	Normal	9·6	6·11	6·0	0·11	10·2	6·3	3·79	6·49 (4·02)	36·6	7·44	..	..	25·1	.. ..	6
7	N. H.	21	M	Normal	9·35	6·31	6·0	0·31	9·96	6·3	3·35	6·76 (4·47)	39·0	7·47	..	..	27·8	.. ..	7
															Urine Ca				
8	R. W.	53	F	Parathyroid adenoma, no bone disease	10·37	7·1	7·0	0·1	11·04	7·35	3·59	6·84 (3·43)	40·0	7·41	235	2·4	25·8	16 days pre-operation	8
					10·28	7·0	6·9	0·1	10·93	7·25	3·58	..	46·1	7·39	..	3·0	28·2	6 „ „	
					9·0	5·43	5·0	0·43	9·55	5·25	3·87	6·25 (3·51)	37·6	7·46	..	3·3	27·1	3 days post-operation	
					9·58	6·15	..	..	10·19	..	3·76	6·68 (3·8)	40·5	7·37	..	3·0	23·7	11 „ „	
															Bl. urea				
9	G. W.	44	F	Parathyroid adenoma, no bone disease	12·95	9·4	9·0	0·4	13·72	9·45	3·87	6·1 (3·5)	30·8	7·43	69	2·3	20·3	Before operation	9
					10·2	6·8	6·9	(−0·1)	10·81	7·25	3·66	..	32·5	7·38	..	4·9	19·5	„ „	
					13·6	9·7	9·45	0·25	14·5	9·93	4·32	..	33·0	..	..	3·0	..	7 days after 1st operation	
					6·9	4·45	3·57	0·88	7·26	3·73	2·65	5·12 (3·36)	29·8	7·29	159	5·6	14·6	6 days after 2nd operation	
10	E. C.	67	F	Parathyroid adenoma, bone disease, renal failure, and stones	11·95	8·62	6·7	1·92	12·7	7·05	3·73	6·4 (4·6)	28·0	7·42	96	4·1	18·6	Pre-operation	10
					12·75	8·75	7·34	1·41	13·55	7·7	4·44	..	27·2	7·47	96	3·4	20·2	Post-operation	
															Urine Ca				
11	E. P.	31	F	Primary hyperparathyroidism with bone disease	13·1	8·8	8·6	0·2	14·02	9·04	4·78	8·4 (5·1)	28·4	7·5	520	1·8	22·3	.. ..	11
12	D. B.	63	F	Parathyroid adenoma, sarcoidosis	11·22	7·4	6·56	0·84	11·95	6·89	4·22	6·7 (3·93)	30·4	7·42	..	4·2	20·0	.. ..	12
13	F. C.	43	F	Post-thyroidectomy, hypoparathyroidism	5·85	4·0	3·53	0·47	6·28	3·71	2·1	8·5 (5·1)	30·3	7·5	6	7·2	23·9	Tetany	13
					8·5	5·4	4·4	1·0	9·06	4·62	3·44	6·84	28·7	..	253	5·0	..	On DHT, no tetany	

* Values in parentheses refer to plasma albumin; other values refer to total plasma protein.
† Plasma inorganic phosphate expressed as mg. phosphorus per 100 ml. plasma.
‡ DHT = dihydrotachysterol.

Case number	Patient	Age (years)	Sex	Main diagnosis	Plasma calcium fractions: Total in plasma	In ultrafiltrate (mg./100 ml.): Total	Ionized	Complexed	In plasma water: Total (mg./100 g.)	Ionized (mg./100 ml.)	Protein-bound (mg./100 ml.)	Plasma proteins* (G./100 ml.)	Alveolar CO_2 (mm. Hg)	pH (Calculated)	Various (Urine Ca mg./24 hr.; blood urea mg./100 ml.) Bl. urea	Plasma: Phosphorus† (mg./100 ml.)	Bicarbonate (m-equiv./l.)	Remarks‡	Case number
14	D. H.	12	M	Type 2 nephritis	8·18	5·98	5·9	0·08	8·50	6·2	2·11	4·92 (1·65)	33·6	7·44	33	4·7	23·2	Not on katonium	14
15	L. W.	41	M	Nephrotic syndrome	8·85	6·18	5·88	0·3	9·25	6·18	2·77	4·5 (2·45)	25·6	7·6	36	3·9	27·3	Bl. urea determined 4 months earlier	15
16	T. W.	13	M	Type 2 nephritis	6·88	4·87	4·7	0·17	7·16	4·94	2·05	5·4 (1·78)	31·7	7·47	39	5·4	23·3	On katonium 30 g. daily, 5 days a week	16
17	L. C.	33	F	Generalized lymphomatosis with renal amyloidosis, nephrotic syndrome	8·5	5·7	4·53	1·17	8·95	4·76	3·02	5·4 (2·5)	27·7	..	216	3·5	13·7	No katonium or steroid therapy	17
18	D. K.	41	M	Type 2 nephritis	5·28	3·88	3·05	0·83	5·56	3·2	1·53	5·3 (2·62)	24·4	7·42	264	15·1	16·1	Plasma Mg 2·2 mg./100 ml.	18
19	U. B.	33	F	Chronic pyelonephritis, chronic renal failure	8·92	5·8	5·63	0·17	9·45	5·92	3·36	6·2 (4·1)	36·4	..	96	5·1	22·0	.. ..	19
20	K. N.	11	M	Chronic glomerulonephritis	5·1	3·3	2·75	0·55	5·43	2·89	1·99	6·6 (4·2)	25·8	7·31	396	12·2	13·4	No vitamin D	20
					6·28	4·2	3·41	0·79	6·68	3·58	2·31	6·5 (3·7)	40·5	7·49	402	9·2	31·3	Plasma Mg 3·04 mg./100 ml.	
21	A. W.	41	F	Hypoplastic kidneys, sec. hyperparathyroidism	8·75	6·3	5·5	0·8	9·28	5·77	2·71	6·1 (3·9)	28·8	7·34	141	4·3	15·9	No vitamin D	21
					9·1	6·55	6·03	0·52	9·65	6·34	2·79	6·12 (3·42)	28·4	7·34	216	6·6	15·8	On vitamin D_2 2 mg. a day	
22	A. C.	15	F	Urinary obstruction, rickets, chronic pyelonephritis, sec. hyperparathyroidism	8·4	5·68	5·6	0·08	8·96	5·88	3·0	7·2	23·7	7·35	123	5·4	13·5	No vitamin D	22
23	S. P.	18	F	Chronic nephritis, sec. hyperparathyroidism (cured)	9·8	6·2	5·35	0·85	10·49	5·62	4·02	7·26 (4·35)	30·0	7·49	174	8·3	22·8	On DHT 0·125 mg. a day	23
					11·25	7·2	6·0	1·2	12·03	6·3	4·53	7·44 (5·0)	34·2	7·41	240	9·1	21·7	„ 0·25 „	
24	G. P.	16	M	Urinary obstruction, rickets, chronic renal failure (cured)	10·55	7·12	6·55	0·57	11·2	6·88	3·75	6·51 (4·34)	35·2	7·44	90	5·3	23·7	.. ..	24
					13·12	8·75	8·32	0·43	13·9	8·73	3·74	6·10 (3·64)	39·0	7·35	156	6·0	22·1	.. ..	
25	V. C.	24	F	Renal tubular osteomalacia type 1 (cured)	9·25	6·22	6·0	0·22	9·85	6·3	3·33	6·80 (3·75)	35·8	7·45	24	2·5	25·3	On DHT 5 mg. a day	25
26	M. F.	57	F	Renal tubular osteomalacia type 1	8·9	6·05	6·0	0·05	9·36	6·3	3·01	5·27 (3·07)	28·2	7·56	27	2·1	26·3	No vitamin D	26
27	K. W.	8	F	Renal tubular rickets, type 2	9·55	5·9	5·65	0·25	10·18	5·93	4·0	7·0	34·8	7·38	27	3·1	20·9	On vitamin D_2 3 mg. a day	27
															Urine Ca				
28	A. G.	30	M	Renal tubular osteomalacia type 2	9·5	6·18	5·9	0·28	10·2	6·2	3·72	7·8	28·3	7·51	49	1·8	22·8	No vitamin D	28
					9·7	7·26	6·0	1·26	10·41	6·3	2·85	..	37·6	7·45	398	2·8	..	On DHT 3 mg. a day	

Case number	Patient	Age (years)	Sex	Main diagnosis	Plasma calcium fractions: Total in plasma	In ultrafiltrate (mg./100 ml.): Total	Ionized	Complexed	In plasma water: Total (mg./100 g.)	Ionized (mg./100 ml.)	Protein-bound (mg./100 ml.)	Plasma proteins* (g./100 ml.)	Alveolar CO_2 (mm. Hg)	pH (Calculated)	Various (Urine Ca mg./24 hrs.; Blood urea mg./100 ml.)	Plasma: Phosphorus† (mg./100 ml.)	Bicarbonate (m-equiv./l.)	Remarks‡	Case number
															Urine Ca				
29	M. E.	51	F	Steatorrhoea, osteomalacia	9·15	5·93	5·5	0·43	9·67	5·77	3·47	5·9 (3·6)	31·3	7·48	4	4·1	23·4	Vitamin D_2 15 mg. a day for 2 weeks	29
					9·28	6·22	5·5	0·72	9·9	5·77	3·41	6·93 (3·92)	30·0	7·51	200	3·9	23·9	Vitamin D_2 15 mg. a day for 5½ months	
30	F. A.	50	F	Steatorrhoea	8·1	5·6	5·0	0·6	8·6	5·25	2·75	6·13 (3·76)	28·0	7·54	52	2·83	24·1	No vitamin D	30
31	H. M.	60	F	Biliary cirrhosis of liver. Steatorrhoea	9·1	5·83	5·72	0·11	9·66	6·0	3·55	6·4 (3·0)	28·9	7·51	..	3·1	23·2	No vitamin D	31
32	I. M.	52	F	Idiopathic osteoporosis	9·35	6·3	6·5	(−0·2)	9·95	6·82	3·33	6·7 (4·5)	37·3	7·4	100	..	23·3	.. ..	32
					9·55	6·42	6·18	0·24	10·11	6·49	3·38	6·1 (3·75)	36·2	7·49	442	..	27·4	DHT 2 mg. a day for a month	
33	W. A.	37	M	Idiopathic osteoporosis	9·7	6·53	6·45	0·08	10·33	6·77	3·48	6·82 (4·42)	37·0	7·41	..	..	24·2	.. ..	33
34	E. E.	60	F	Senile osteoporosis	9·08	6·2	5·33	0·87	9·66	5·6	3·19	6·72 (3·57)	37·0	7·36	170	3·1	21·7	.. ..	34
35	K. S.	36	M	Idiopathic hypercalcuria, renal stones	9·77	6·23	6·05	0·18	10·45	6·35	3·92	7·5	36·8	7·47	580	..	26·7	.. ..	35
36	J. A.	40	M	Idiopathic hypercalcuria, renal stones	9·28	6·1	6·0	0·1	9·9	6·3	3·5	7·0	35·7	7·4	350–400	..	22·6	.. ..	36
37	T. L.	34	M	Idiopathic hypercalcuria, renal stones	9·05	6·4	5·77	0·63	9·65	6·05	2·97	7·1 (4·6)	42·0	7·38	270	2·7	24·9	.. ..	37
38	M. H.	16	M	Idiopathic tetany	9·45	6·07	5·65	0·42	10·05	5·93	3·7	6·5	30·1	7·46	..	..	21·7	.. ..	38
				overbreathing	10·13	5·65	4·51	1·14	10·8	4·73	4·93	6·9	9·3	7·98	..	..	21·7	.. ..	
															Plasma K (m-equiv./l.)				
39	R. B.	47	M	Idiopathic tetany	9·2	6·0	5·9	0·1	9·8	6·2	3·5	6·77 (4·07)	43·5	7·37	4·1	..	25·8	.. ..	39
				overbreathing	9·6	5·58	5·21	0·37	10·21	5·47	4·37	..	19·0	7·76	4·4	..	26·4	Plasma Mg 2·3 mg./100 ml.	
40	S. I.	42	F	Idiopathic tetany	9·3	6·07	5·6	0·47	9·95	5·88	3·6	7·4 (4·78)	34·1	7·46	3·9	..	24·4	„ 2·11 „	40
				overbreathing	9·35	5·5	4·85	0·65	10·0	5·09	4·26	..	15·7	7·78	4·2	..	23·2	„ 2·17 „	
41	S. W.	36	F	Idiopathic tetany	8·7	5·9	5·8	0·1	9·32	6·09	3·13	7·6 (4·3)	34·4	7·46	..	..	24·5	.. ..	41
				overbreathing	9·9	5·75	4·81	0·94	10·6	5·05	4·61	..	19·0	7·72	5·2	..	24·0	.. ..	
42	J. W.	27	F	Idiopathic tetany. Turner syndrome	8·9	5·97	5·67	0·3	9·46	5·95	3·21	6·48	26·0	7·46	3·8	..	18·3	Plasma sodium 153 m-equiv./l.	42
															Urine Ca				
43	L. R.	47	F	Vit. D deficiency osteomalacia (cured)	9·07	6·1	6·1	0·0	9·66	6·4	3·26	6·7	36·0	7·54	260	..	30·9	Vitamin D_2 0·25 mg. a day	43
44	G. P.	9	F	Hypo-hyperparathyroidism	10·65	7·1	5·88	1·22	11·4	6·17	4·01	7·3 (4·1)	30·2	7·52	325	5·2	24·8	Vitamin D_2 3 mg. a day	44
					14·18	8·9	7·83	1·07	15·1	8·22	5·75	6·93 (3·96)	28·7	7·56	404	5·1	25·1	Vitamin D_2 3 mg. a day (intoxicated)	

* Values in parentheses refer to plasma albumin; other values refer to total plasma protein.
† Plasma inorganic phosphate expressed as mg. phosphorus per 100 ml. plasma.
‡ DHT = dihydrotachysterol.

the Clinical Pathology Department of the hospital. Plasma proteins were determined either in the same department, or by Miss V. J. Patston, by the standard micro-Kjeldahl technique, with precipitation of globulin by 42 per cent. sodium sulphite. Various other determinations were carried out at the Metabolic Ward Laboratory. Plasma pH was determined by calculation, using the Henderson–Hasselbalch equation: pH is $6{\cdot}1+\log \frac{H.HCO_3}{HCO_3^-}$

In some cases serial determinations were carried out after raising and lowering the plasma-phosphorus level by means of oral sodium phosphate (pH 7·4) or aluminium hydroxide respectively. Results from these experiments are shown only where they are of special relevance to the present paper. The inferences to be drawn from studying the effect of plasma phosphorus on the plasma calcium will be reported later by G. R. Philpot.

Induction of alkalosis by overbreathing. Each of six subjects was asked to overbreathe for approximately 10 minutes. At the end of this period an alveolar air sample was taken and venous blood collected. For technical reasons we were unable to carry out more than one determination of ionized calcium on any one day, so that the control measurements in the same subjects were carried out on another day. The time interval was generally one week, but was one day only in Case 41, and as long as three weeks in Case 1 (a normal subject). One of the two normal subjects so investigated was an adult medical student. The other was a doctor. The four patients (Cases 38 to 41) had idiopathic tetany, and are described below and in the Appendix.

All results of plasma-phosphorus determinations refer to the inorganic phosphate only, and are expressed as mg. of elementary phosphorus in 100 ml. of plasma.

Results

The results of determination of the plasma calcium fractions, with other data, are shown in the Table, which includes the majority of the results obtained up to July 1957. Plasma-calcium measurements quoted in this Table, and elsewhere (except the Appendix), refer to the content in 100 ml. of plasma water unless otherwise stated. Such results are slightly higher than when expressed in terms of 100 ml. of plasma. The plasma ionized calcium levels in seven normal men were 5·88 to 6·51 mg. per 100 ml., and are shown in Fig. 1, where the normal plasma calcium fractions obtained in the present work are compared with those obtained by Rose (1957). The Table shows the results of 66 determinations of plasma calcium fractions from the seven normal men and from 37 patients with a variety of diseases known to affect calcium metabolism. The concentration of ionized calcium in plasma water ranged from 2·89 mg. to 9·93 mg. per 100 ml., and the distribution of these values in relation to the various diseases is shown in Fig. 2. The results of serial studies of two patients with parathyroid adenomata are shown in Fig. 3; studies of patients with the nephrotic syndrome are illustrated in Fig. 4. The responses to overbreathing in normal subjects

and in patients with idiopathic tetany are shown in Fig. 5. A study of the relation of plasma ionized calcium to tetany is shown in Fig. 6. In the seven normal men the values for the plasma complexed calcium were 0·11 to 0·41 mg. per 100 ml., and for the plasma protein-bound calcium 3·35 to 3·93 mg. per 100 ml. These normal values are shown in Fig. 1. Failures were very few. The ultrafiltration shell broke on two occasions only; then the plasma was recovered,

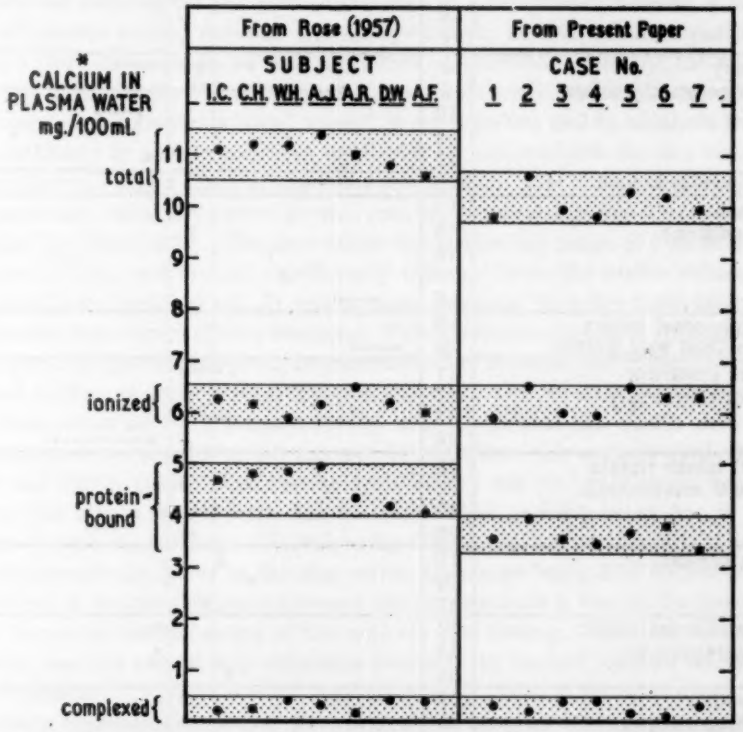

FIG. 1. Plasma calcium fractions as determined in seven normal subjects by Rose (1957) and in the seven normal subjects reported in the present paper. The different range of the protein-bound fractions in the two series corresponds with the difference in the range of total plasma calcium, attributable to a change in method. The earlier work used an oxalate method, whereas the present work used EDTA titration (see text). The normal ranges of ionized and complexed calcium fractions in the plasma remain unchanged.

* Our figures, expressed per 100 mg. of plasma water, give slightly higher readings than if expressed per 100 mg. of whole plasma.

re-equilibrated with the appropriate carbon-dioxide tension, and ultrafiltration resumed with a new shell. In two cases the plasma ionized calcium was found to be greater than the ultrafilterable fraction. This obvious error, however, did not exceed 0·2 mg. per 100 ml., representing the limit of expected error of the methods.

Discussion

It is necessary to consider whether we have, by the present techniques, been

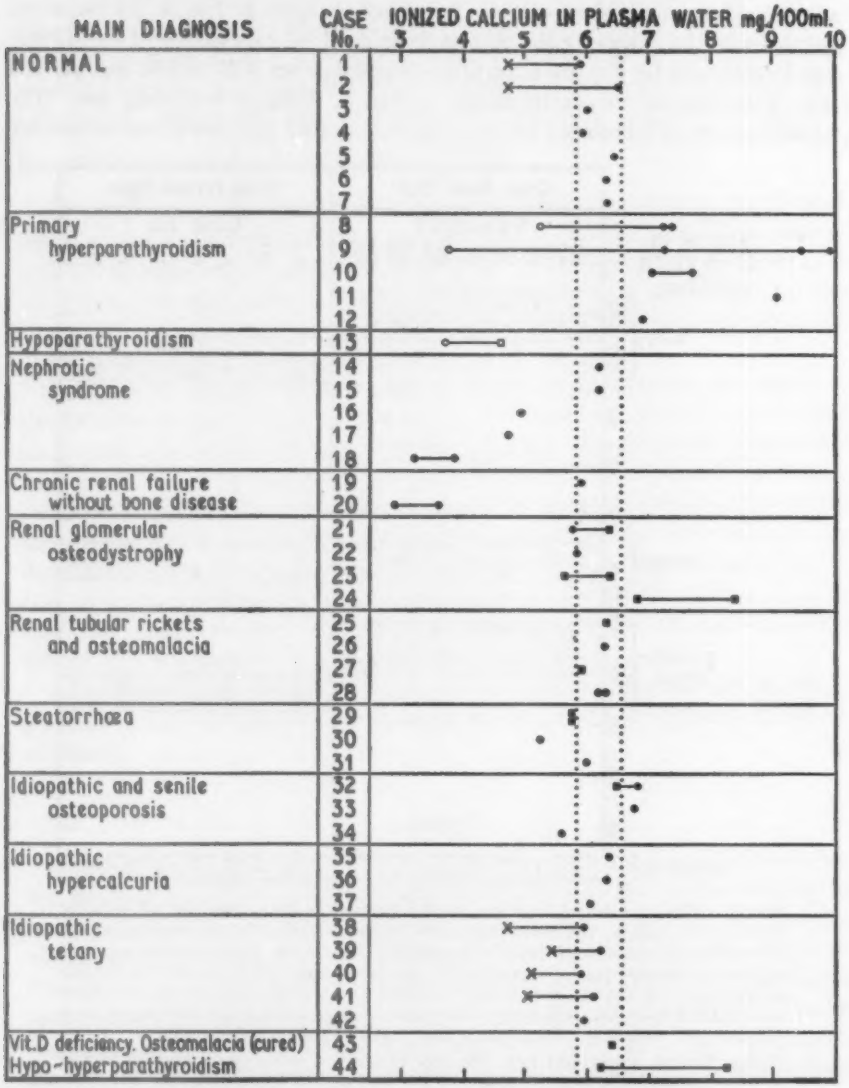

FIG. 2. Values of plasma ionized calcium in seven normal men and in 37 patients with various diseases. Continuous lines join the values obtained in the same subject on different days.

× : after overbreathing. ○ : after removal of parathyroid glands or adenoma.
□ : treated with a form of vitamin D after removal of parathyroid glands or adenoma.
■ : treated with a form of vitamin D (D_2, D_3, or dihydrotachysterol).
● : remaining values.

able to measure the plasma ionized calcium in the various pathological conditions concerned. There are several pieces of evidence to support the contention that this was so. First, where it would be generally accepted that the plasma ionized calcium level would be low, as in hypocalcaemic tetany following surgical removal of the parathyroid glands (Case 13), a low value was observed. Secondly, in those patients (Cases 9, 11, and 10) who had hypercalcaemia and unequivocal radiological and clinical evidence of hyperparathyroidism, a high level of plasma ionized calcium was always found. In two of these patients (Cases 9 and 10) the diagnosis was confirmed at operation. Thirdly, the value for complexed calcium (the difference between the ultrafiltrable and the ionized components) was generally small, except in renal failure and in alkalosis from overbreathing; in these conditions explanations are available for the consistently observed high values, and are discussed below. The values obtained for plasma ionized calcium in seven normal men are compared in Fig. 1 with those obtained by Rose (1957). The new values fall within the range of 5·88 to 6·51 mg. per 100 ml., and are not significantly different from the earlier values of 5·93 to 6·24 mg. per 100 ml. It will be seen, however, that the total plasma-calcium levels are lower in the new series. This is because the earlier values were determined by the oxalate-precipitation method of Kramer and Tisdall (1921), whereas in the new series EDTA titration was used. Our results therefore agree with those reported by Wilkinson (1957), who found that the EDTA method gave results lower by 0·7 to 0·8 mg. per 100 ml. than the oxalate method of Clark and Collip (1925). The results with EDTA are probably more nearly correct, the higher results from the oxalate method perhaps being due to inclusion of some magnesium. The values for the plasma protein-bound calcium are correspondingly lower in the new series, the range being 3·35 to 3·93 mg. per 100 ml. A further difference between the two methods is that in the present series, but not in the first series, all the subjects were fasting. This circumstance, however, has not caused any difference between the ionized calcium levels in the two series.

Primary hyperparathyroidism. Five undoubted cases have been investigated. Parathyroid adenomata were removed at operation in three cases (Nos. 9, 10, and 8) and the operation was followed in each case by a fall in plasma ionized calcium to normal or subnormal levels. In E. C. (Case 10) this fall was not measured, but was inferred from observation of a striking decrease in the total plasma calcium, together with the onset of tetany, which was relieved by intravenous calcium gluconate. In each of these three cases the tumour was removed, and identified histologically as a chief-cell parathyroid adenoma. In the fourth patient (E. P., Case 11) no adenoma has so far been found, in spite of three explorations of the neck and mediastinum. The diagnosis is firmly based, however, on the biochemical findings and the characteristic radiological signs of hyperparathyroidism in the bones. In the fifth patient (D. B., Case 12) a double diagnosis was made *post mortem*, when a parathyroid adenoma was found in addition to sarcoidosis, thus explaining some difficult features in this case. In all five patients the plasma ionized calcium levels were high (6·9 to

9·9 mg. per 100 ml.). Similar high values have been observed in three other cases of parathyroid adenoma not described in the present paper. The view now held in this laboratory is that the plasma ionized calcium will be found to be increased in cases of primary hyperparathyroidism, even though the total

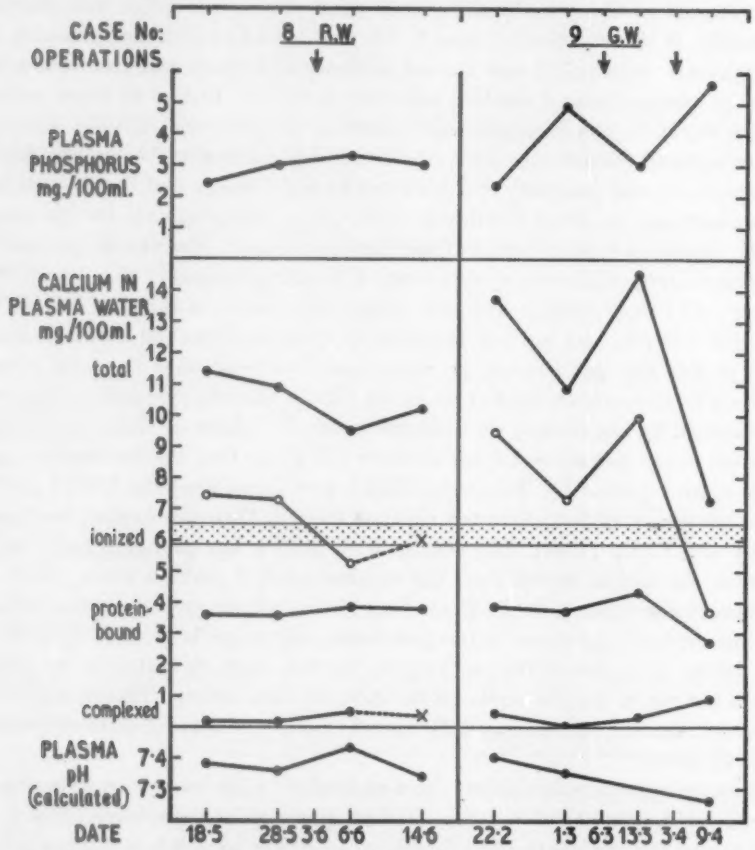

FIG. 3. Plasma calcium fractions and other data in two cases of primary parathyroid adenoma before and after removal of the adenomata. The arrows indicate exploratory operations; the adenoma was found at the first operation in Case 8, but only at the second operation in Case 9.

○: measured values of plasma ionized calcium.

×: plasma ionized calcium not measured directly, but obtained by subtracting plasma complexed calcium (assumed unchanged) from the observed total ultrafilterable calcium.

plasma calcium may be normal, as in one of the patients reported here (R. W., Case 8), in whom the total plasma calcium, as determined in the Metabolic Ward Laboratory, varied from 10·3 to 11·2 mg. per 100 ml. Diagnosis in this case was particularly difficult, since the plasma phosphorus was also sometimes normal when the patient was untreated (2·7 to 3·4 mg. per 100 ml.), the bones were normal clinically and radiologically, and the plasma alkaline phosphatase

was normal. The cause of the presumed past hypercalcuria and renal stone formation was thought to be either idiopathic hypercalcuria or primary hyperparathyroidism, and the finding of a high level of plasma ionized calcium was taken as strong evidence in favour of the latter diagnosis and against the former (see below). The finding of a diminished protein-bound calcium fraction requires some explanation. It appears to have been due to a somewhat low level of plasma albumin and a slightly low plasma pH (see Table). After removal of the parathyroid adenoma the protein-bound calcium increased slightly (Fig. 3), in spite of a decrease in the ionized fraction. The increase in the protein-bound fraction may be accounted for by the rise in the level of plasma albumin and in pH which then occurred. The plasma ionized calcium became subnormal on the third day after operation, and normal by the 11th day. Inspection of the data from some of the other cases reveals some points of interest. In one patient (G. W., Case 9), who had bilateral renal calculi and no bone disease, the protein-bound plasma calcium was again less than might have been expected. This may again be attributed to a low plasma-albumin level and pH. Thus a very high ionized-calcium level (9·5 mg. per 100 ml.) was accompanied by a protein-bound calcium level of 3·9 mg. per 100 ml., which is just within normal limits. After removal of the adenoma the ionized calcium level fell to 3·7 mg. per 100 ml. (see Fig. 3), and tetany appeared, while the protein-bound calcium decreased by a relatively much smaller amount, to 2·7 mg. per 100 ml. A part of this fall must have been due to the fall in plasma pH which followed operation. We therefore conclude that the binding of calcium by the plasma protein is decreased by parathyroid hormone.

In E. C. (Case 10) the radiological changes in the bones were pathognomonic of hyperparathyroidism, and the biochemical findings left little doubt that this condition was primary, and that renal failure was secondary. The level of total plasma calcium was high, blood urea was increased, and plasma phosphate was normal. The level of plasma alkaline phosphatase was grossly elevated. The high concentration of plasma ionized calcium (7 to 7·7 mg. per 100 ml.) was taken to confirm the diagnosis of primary rather than secondary hyperparathyroidism. Plasma protein-bound calcium was normal (3·7 mg. per 100 ml.) when the pH was 7·39, and increased (4·4 mg. per 100 ml.) when the pH was 7·44. The complexed calcium of the plasma was twice noted to be remarkably increased. This is thought to have been due mainly to the renal failure (discussed further below), since we have not observed such high values in the patients with parathyroid adenoma who did not have severe renal failure. The patient died on the third day after operation from a large myocardial infarction, so that no post-operative estimation of plasma ionized calcium was made. There was no doubt, however, that its level was subnormal (see above).

In E. P. (Case 11) there was no doubt about the diagnosis (see above, and the Appendix), although the adenoma has not yet been found. The plasma ionized calcium was much increased (9 mg. per 100 ml. of plasma water), whereas the protein-bound fraction was only slightly increased (4·8 mg. per 100 ml.). There was no acidosis, the blood urea was normal, and the total plasma proteins were

slightly increased, with a normal electrophoretic strip. We again conclude that the excess of parathyroid hormone has caused a fall in the calcium-binding power of the plasma proteins. In Case 12 the history was very suggestive of sarcoidosis, and this diagnosis had been confirmed by skin biopsy some years previously. When the patient developed hypercalcaemia it was attributed to sarcoidosis, and further evidence for this view was the elevation of the plasma-calcium level which followed a small dose of calciferol. We were surprised to note, however, that the plasma-calcium level could not be made to fall to normal by giving cortisone, since such a fall is known to occur in uncomplicated sarcoidosis, but does not occur if the hypercalcaemia is due to hyperparathyroidism (Dent, 1956). There were no other reasons for diagnosing primary hyperparathyroidism *ante mortem*, and further investigations were delayed on account of the urgent necessity for surgery to relieve impending glaucoma. The patient subsequently became uraemic, and died, and at autopsy was found to have both sarcoidosis and a chief-cell parathyroid adenoma.

Hypoparathyroidism. In Case 13 the history of the patient (F. C.) (see Appendix) left little doubt that her tetany was due to removal of all the parathyroid glands at the time of thyroidectomy, and that it could be accounted for entirely by the diminution of plasma ionized calcium (3·7 mg. per 100 ml.). After treatment with dihydrotachysterol (6 mg. a day) the ionized calcium level rose to 4·6 mg. per 100 ml., and the tetany disappeared. At the same time the plasma complexed fraction rose from the normal value of 0·47 mg. to the abnormal value of 1·0 mg. per 100 ml., and the 24-hour urine calcium increased from 6 mg. a day to 253 mg. a day. The increase in the complexed fraction may well have been due to a rise in the level of plasma citrate, which is known to occur with vitamin-D therapy (Harrison and Harrison, 1952; Harrison, 1954, 1956). We were impressed by the magnitude of the increase in urine calcium at a time when the plasma ionized calcium was still subnormal, and we consider that this increase may have been directly due to the increase in the complexed fraction, and that the main complexing agent responsible may be citrate.

Nephrotic syndrome. Five patients have been investigated. The blood-urea level was normal in three, but elevated in the other two. All had low total plasma-calcium levels (5·6 to 9·3 mg. per 100 ml.), having been selected for investigation for this reason. The results, illustrated in Fig. 4, show that in two of the patients without uraemia the plasma ionized fraction was normal, and that the low total concentration of calcium in the plasma was entirely due to a diminished protein-bound fraction associated with a low level of plasma protein. Further, the protein-bound calcium appears to be proportional to the plasma albumin. Of the three patients in whom the plasma ionized calcium was diminished, two had uraemia, but the third did not. We are unable to suggest what factors cause depression of the ionized fraction in the latter class of patients. It has not appeared to correlate constantly with the blood-urea level, plasma phosphate, or pH. Tetany has been described, in cases of the nephrotic syndrome uncomplicated by renal failure or phosphorus retention, by Tegelaers and Tiddens (1955) and Stanbury and Macaulay (1957). The latter authors

believed, with good circumstantial evidence, that the tetany of their patient was due to a low level of plasma ionized calcium. Such evidence was lacking in the case described by Tegelaers and Tiddens, but a decrease of plasma ionized calcium may occur in the uncomplicated nephrotic syndrome more frequently than has been suspected, and has been observed recently in two other cases not reported in the present series. The complexed calcium fraction was increased in the two patients with renal failure, as was found in other cases of renal failure (see above and below).

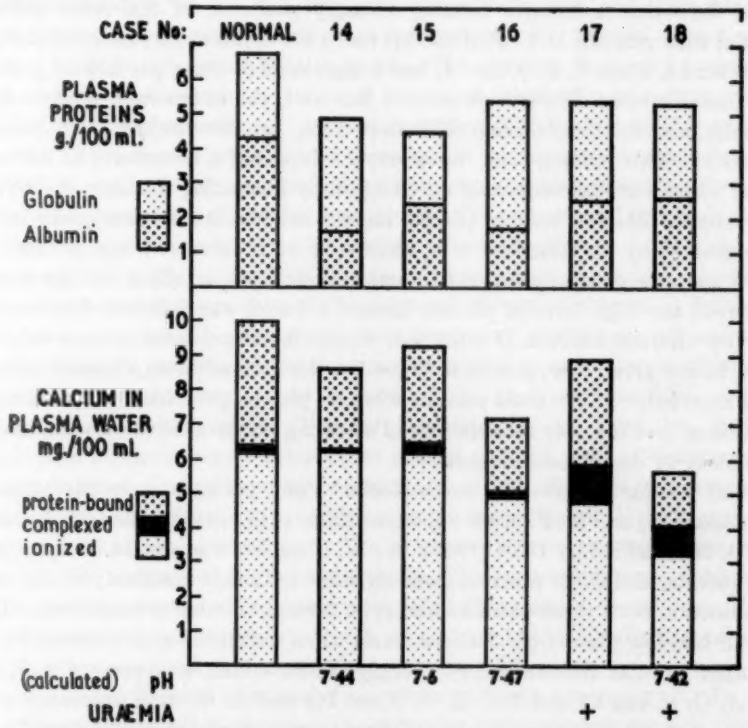

FIG. 4. Plasma proteins and calcium fractions in five cases of the nephrotic syndrome with and without raised blood-urea levels. In Cases 14 and 15, in which the ionized calcium fraction is normal and the blood urea is also normal, the protein-bound calcium appears to be proportional to the plasma albumin. The diminished plasma ionized calcium in Case 16 was not expected since the blood urea and plasma phosphorus were both normal. Plasma complexed calcium is increased in the cases of uraemia.

Renal glomerular osteodystrophy (Azotaemic osteodystrophy). Results in five cases are reported. All five patients had severe renal damage, with high blood-urea levels. The radiological appearances of the bones before treatment varied from almost pure secondary hyperparathyroidism in A. W. (Case 21) to almost pure rickets in G. P. (Case 24). All, however, had severe bone disease, except K. N. (Case 20), in whom only minimal signs of hyperparathyroidism could be observed. Two (K. N. and A. W., Cases 20 and 21) were untreated with

vitamin D when the plasma ionized calcium was first measured, and the values in these cases were very low and slightly low respectively. The current view in this department is that in cases of this type the level of plasma ionized calcium is normal or low in the untreated state, and that the finding of a high level indicates either primary hyperparathyroidism (or secondary parathyroid adenoma) or excessive vitamin-D therapy. Further, one of the objects in treatment with vitamin D is to maintain the plasma ionized calcium within normal limits. Thus, in A. W. (Case 21), the plasma ionized calcium increased to normal while the calcium balance became strongly positive; of the other patients treated with vitamin D, S. P. (Case 23) had a low ionized calcium level (5·6 mg. per 100 ml.), while G. P. (Case 24) had a high level (6·9 mg. per 100 ml.), at the first observations. The former patient felt well, the bones were healed radiologically, and the total plasma calcium (9·8 mg. per. 100 ml.) revealed no cause for alarm. Two months later, however, she reported a recurrence of her bone pains, which were subsequently cured again by increasing the dose of dihydrotachysterol. The low level of plasma ionized calcium on the first occasion had been masked by the increased complexed calcium fraction (0·8 mg. per 100 ml.) which we have generally found in renal failure. In G. P. (Case 24) the reverse occurred; the high level of plasma ionized calcium was followed four months later by obvious vitamin-D intoxication and increased total plasma calcium. Thus, in any given case, attempts to predict the level of plasma ionized calcium from knowledge of the total plasma calcium, plasma proteins, and pH may be hazardous, and the only reliable way of assessing the level of the plasma ionized calcium is by direct measurement.

Renal tubular rickets and osteomalacia. Four patients were investigated. V. C. (Case 25) and M. F. (Case 26) were adults with renal tubular osteomalacia type 1, as classified by Dent (1952). K. W. (Case 27) was a child of eight years with rickets, and A. G. (Case 28) was an adult with osteomalacia; by the same classification both these patients had type 2 renal tubular osteomalacia. Thus all four had low plasma-phosphorus levels when untreated with vitamin D, but no other tubular defects except the glycosuria which was present in K. W. and A. G. (Cases 27 and 28). M. F. (Case 26) and A. G. were untreated when the plasma ionized calcium levels were first measured, and they were found to be normal in this respect. In the treated patients the same state of affairs was found. Our present view, in agreement with that of Dent and Harris (1956), is that a low level of plasma ionized calcium is not found in patients with these varieties of osteomalacia, in contrast with renal glomerular rickets or osteomalacia, in which the plasma ionized calcium may be reduced (see above). This is of some importance in view of the theories held elsewhere (Albright and Reifenstein, 1948) that the decrease of plasma phosphorus in such cases is due to a secondary hyperparathyroidism following stimulation of the parathyroids by 'a tendency to a low serum calcium'. It is difficult in our cases to find any tendency to diminution of the plasma ionized calcium, this being the fraction we should consider to be active in this context.

Steatorrhoea. Results obtained from three patients are reported, in each of

whom the diagnosis had been fully confirmed by six-day fat-balance studies. In H. M. (Case 31) the steatorrhoea followed the onset of jaundice from primary biliary cirrhosis. In contrast to the other patients who had definite or possible idiopathic steatorrhoea, she showed no signs of metabolic bone disease clinically, radiologically, or by the calcium balance, which was normal in all respects. The plasma total calcium was reduced, but study of the calcium fractions revealed a normal ionized fraction, the low total being wholly attributable to a decrease of plasma proteins. M. E. (Case 29) had severe osteomalacia, proved clinically and radiologically. On a gluten-free and high-calcium diet, and large doses of calciferol (15 mg. intramuscularly once a fortnight), she showed only a small positive calcium balance of 130 mg. a day. This positive balance increased to 1·4 g. a day with the enormous dose of 15 mg. of calciferol a day given orally, and the level of plasma ionized calcium was then slightly low at (5·8 mg. per 100 ml.). After five months of this therapy the positive calcium balance was maintained, and the plasma ionized calcium remained at the same level, although the plasma complexed calcium increased from a normal to a high value. We think it significant that the patient in whom the plasma ionized calcium was normal had a normal calcium balance, whereas the patient with a low level of plasma ionized calcium showed the calcium balance of vitamin-D deficiency and gross osteomalacia. The total plasma-calcium levels were virtually the same in these two cases, and the determination of plasma ionized calcium may prove to be a good screening test in steatorrhoea, to determine which patients are developing osteomalacia. The third patient, F. A. (Case 30), had no radiological signs of bone disease other than a slightly rarefied skeleton, and calcium-balance studies were not carried out. Clinically, the only symptom which could be attributed to a syndrome mimicking vitamin-D deficiency was a generalized muscular weakness. The interpretation of this weakness, however, remains in doubt at the time of writing. The urine calcium was diminished (approximately 40 mg. a day), suggesting that there was a disturbance of calcium metabolism, an impression which was supported by the finding of a low level of plasma ionized calcium (5·3 mg. per 100 ml.).

Osteoporosis. Results obtained from three patients are reported. In each case there seemed no doubt that the difficult diagnosis was correct. It was based on a clear history of loss of height, fractures of decalcified brittle bones, and the finding of no biochemical abnormality other than an increase of calcium in the urine. Cases 32 and 33 (I. M. and W. A.) were regarded as idiopathic; E. E., the third patient (Case 34), had senile osteoporosis. It has generally been assumed that the plasma ionized calcium, if measured in such cases, would prove to be normal. Our results, however, show some departures from normal. In the one patient with senile osteoporosis (Case 34) the plasma ionized calcium was decreased (5·6 mg. per 100 ml.), while in one of the patients with idiopathic osteoporosis (Case 33) the second value obtained for the plasma ionized calcium was at the upper limit of normal, whereas the first was distinctly above normal, but under suspicion of error, since it was apparently higher than the plasma ultrafilterable calcium.

Idiopathic tetany. Four patients (Cases 38, 39, 40, and 41) who had a condition similar to that described by McCance (1932) have been investigated. Each had tingling sensations or cramps at different times, and showed either a positive

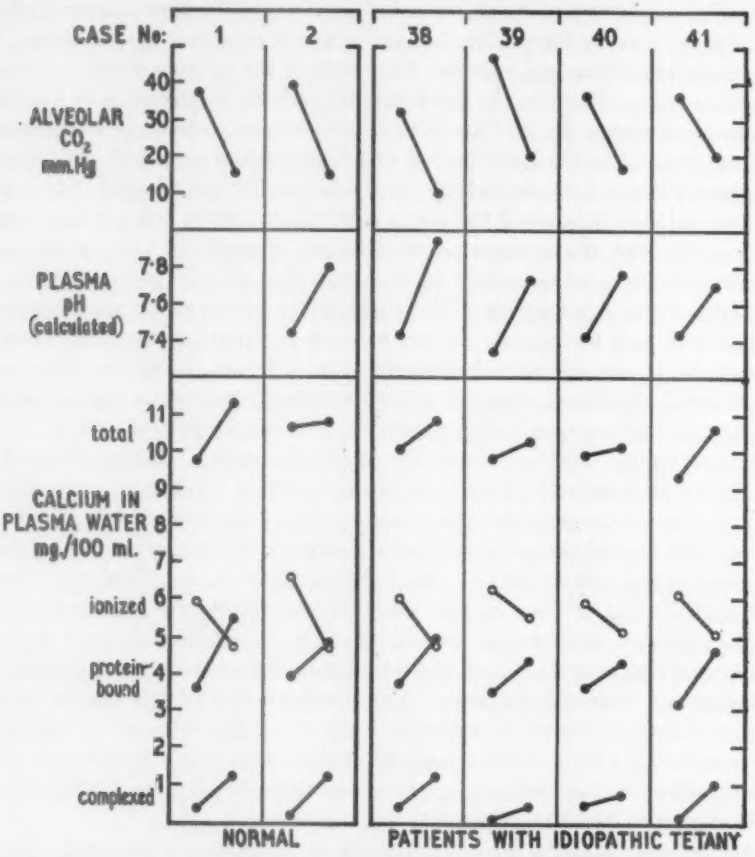

FIG. 5. Plasma calcium fractions in two normal men and in four patients with idiopathic tetany, before and after overbreathing. In each case overbreathing is accompanied by a decrease in the ionized fraction, despite an increase in the total plasma calcium. The decrease in the ionized fraction is accompanied by increase in both the protein-bound and the complexed fraction, each accounting for approximately half of the decrease in the ionized fraction.

○: plasma ionized calcium.
●: all other values.

Chvostek sign or a positive Trousseau sign, or both. No biochemical abnormalities could be found in the plasma of the patients when not overbreathing, except that on one occasion in Case 41 the plasma calcium was as low as 9·3 mg. per 100 ml. owing to a slight diminution of the protein-bound fraction. Results of the overbreathing experiments are shown in Fig. 5. In each case the ionized and other fractions of calcium in the plasma were normal initially (except the low protein-bound fraction in Case 41). The falls in alveolar carbon-

dioxide tension were followed by approximately the same changes in the plasma calcium fractions in the normal subjects and in the patients. In each there was a slight increase in the total plasma calcium, which is probably significant in spite of the time intervals involved, since it occurred in every case, and since a similar rise was found by McCance (1932) in one of his two cases, and by Grant and Goldman (1920) in three normal subjects after overbreathing. The level of plasma ionized calcium fell in each of the present cases by 0·7 to 1·8 mg. per 100 ml. This fall was accompanied by an increase in the protein-bound calcium attributable to the increase in pH, and also by an increase, similar in extent, in the complexed calcium fraction, which rose to levels as high as 1·2 mg. per 100 ml. Bock, Dill, and Edwards (1932) reported that overbreathing for about 25 minutes was regularly followed by an increase of blood lactic acid to as much as 28 mg. per 100 ml. Giebisch, Berger, and Pitts (1955) demonstrated a similar rise in the plasma-lactate level in dogs which were made to overbreathe very soon after nephrectomy. Boucot, Lumb, Mahler, and Stanbury (1956) found that the plasma-lactate and plasma-pyruvate levels rose in normal human subjects after they had overbreathed sufficiently to reduce the arterial plasma carbon dioxide by 5 m-moles per litre. Giebisch, Berger, and Pitts (1955) considered that other organic acids might also be increased in the plasma when alkalosis was induced by overbreathing. We suggest that the rise in the level of plasma complexed calcium which followed overbreathing was due to an increase of those plasma organic acids which, like citrate, are able to complex calcium ions.

Overbreathing produced more distress in the patients, with exacerbation of their symptoms, so that they were reluctant to overbreathe to the same extent as the normal subjects; nevertheless, more severe symptoms resulted. The normal subjects developed paraesthesiae and a positive Chvostek sign, whereas the Trousseau sign was not elicited. One of the patients, M. H. (Case 38), developed a very marked Chvostek sign; another, R. B. (Case 39), also experienced 'cramp' of the arms and face; S. I. (Case 40) developed severe carpopedal spasm with elbow flexion, and S. W. (Case 41) carpopedal spasms, trismus, laryngismus stridulus, and abdominal pain. Since these patients showed no abnormality of distribution of calcium in the plasma, and overbreathing caused no more reduction of the ionized calcium than that which occurred in the normal subjects under similar conditions, the possibility of tetany due to deficiency of plasma magnesium was considered. In R. B. and S. I. (Cases 39 and 40) the plasma magnesium was determined for us by Miss D. Bryant, of Epsom District Hospital, and the values she found were slightly high and normal respectively (see Table). S. W. (Case 41) received 24 ml. of a 10 per cent. solution of magnesium sulphate intravenously in 10 minutes. This was sufficient to increase the extracellular-fluid magnesium by 2 mg. per 100 ml., and did not affect the Trousseau sign, which remained positive. It is therefore considered most unlikely that magnesium deficiency in the extracellular fluid could have been the cause of the tetany in these cases. Its true cause remains unknown, and it may be necessary to investigate the intracellular biochemistry, since Fourman and

McCance (1955) have found that tetany may be associated with a high intracellular total cationic level.

Another patient, J. W. (Case 42), also had tetany, but it was associated with gonadal agenesis and osteoporosis (the patient described by Geffen (1956)). She was not subjected to the overbreathing test, but had been observed to overbreathe spontaneously if nervous, when the muscular spasms in her arms and legs became very severe. On her second admission the plasma-calcium and plasma-potassium levels were low (see Appendix), whereas both these values had been normal at the first admission. Tetany was present on both occasions, but when the plasma ionized calcium was determined on her second admission it was normal. Nevertheless, treatment with dihydrotachysterol improved her tetanic symptoms, without actually increasing the total plasma calcium (although intoxication and hypercalcaemia occurred later). A similar anomalous relief of normocalcaemic tetany by dihydrotachysterol has been reported by Sandock (1956). In his patient no cause was found for the tetany, which was, however, noted almost always to occur in the week before menstruation. In J. W. no consistent cause was found for the tetany; plasma magnesium was determined on the patient's first admission, and was found to be normal.

Role of Plasma Ionized Calcium in Tetany

Fig. 6 shows the relationship between the incidence of tetany and the levels of plasma ionized calcium in the cases now to be discussed. In patients with hypoparathyroidism, either surgically induced, as in F. C. (Case 13), or functional but temporary after removal of a parathyroid adenoma, as in G. W. (Case 9), tetany occurred when the plasma ionized calcium was less than 4·3 mg. per 100 ml., but did not occur when the level was above this figure. When the normal subjects were made to overbreathe, signs of tetany could be elicited when the plasma ionized calcium was above this critical figure, but these cases are not strictly comparable with those shown in Fig. 6. In the former there was a sudden severe alkalosis, and the fall in the level of plasma ionized calcium took place in a few minutes rather than in a few days. Such a fast rate of fall probably increases the likelihood that tetany will result. In all the patients with idiopathic tetany the plasma ionized calcium was normal, as has been pointed out. In two cases of renal failure shown in Fig. 6 the plasma ionized calcium was well below the 'tetany' level, without the occurrence of tetany. It has generally been considered that the hypocalcaemia of renal failure does not cause tetany because the acidosis has a protective effect. This protection has been thought to occur in two ways. First, the low pH of the plasma might cause a decrease in binding of calcium by plasma proteins. In the cases here described, however, it has been shown that the plasma ionized calcium levels were low, so that this argument does not apply. Secondly, a low plasma pH has been thought to protect the nerves from irritability due to low levels of plasma ionized calcium. Again this argument does not apply in K. N. (Case 20), since the patient was accidentally given an overdose of sodium bicarbonate

and made alkalotic, with a plasma pH of 7·46, and even then he had no signs or symptoms of tetany. Hence we are forced to conclude that there is an unknown factor which may protect cases of hypocalcaemic renal failure from tetany. In Case 20 Miss D. Bryant kindly determined the plasma magnesium for us, and found it to be abnormally increased (see Table). It may be that the high level of plasma magnesium was exerting an antitetanic action in this case.

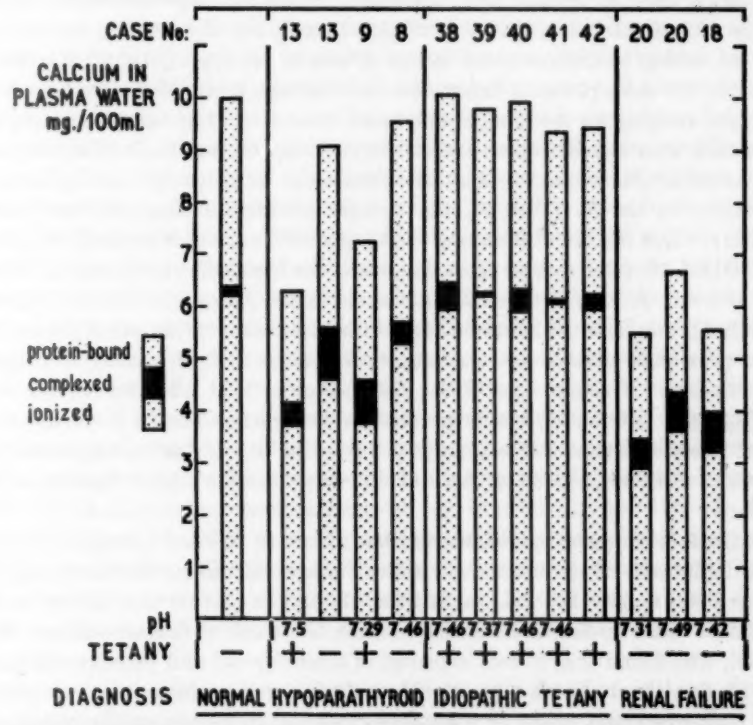

FIG. 6. Plasma calcium fractions in patients with either diminished plasma ionized calcium or tetany, or both. The tetany of hypoparathyroidism develops when the plasma ionized calcium is below a critical value of about 4·3 mg. per 100 ml. In idiopathic tetany the plasma ionized calcium is normal. In renal failure tetany did not occur even with the levels of plasma ionized calcium well below 4·3 mg. per 100 ml., and even with alkalosis in case 20.

Plasma Complexed Calcium

The values found for plasma complexed calcium in normal subjects by Rose (1957) were equal to or less than 0·5 mg. per 100 ml., and the normal values found in the present series are not significantly different. It must be remembered that the complexed calcium fraction is obtained by taking the difference between the total ultrafilterable and the ionized fraction in the ultrafiltrate. Since each of these values is subject to a possible experimental error of approximately 0·1 mg. per 100 ml., it follows that low values for the complexed

fraction may not be very accurate, and that where very low values are found the results may not be significant at all. When, however, the complexed fraction was observed to be over 0·6 mg. per 100 ml., the value has been considered significantly high. High levels of the plasma complexed calcium were regularly found in two conditions. First, in five out of the six subjects investigated in alkalosis after overbreathing, the plasma complexed calcium increased to abnormally high values (see p. 479, the Table, and Fig. 5), and the possible reason for this has already been discussed. Secondly, data are shown relating to the 10 patients who had levels of blood urea near or exceeding 100 mg. per 100 ml. owing to chronic renal failure (Cases 9, 10, 12, 17, 18, 20, 21, 22, 23, and 24). All these patients except two showed high levels of plasma complexed calcium, ranging up to 1·9 mg. per 100 ml. (Case 10). The exceptions are A. C. (Case 22), who appears quite unusual in this respect, and G. P. (Case 24), who had considerable renal tubular disturbances in addition to renal glomerular damage. In G. W. (Case 9), the complexed calcium fraction was normal initially, when the blood urea was 69 mg. per 100 ml., but increased to 0·88 mg. per 100 ml. after the second operation, when the blood-urea level rose to 159 mg. per 100 ml. A high level of organic acids in the plasma is already believed, on other grounds, to occur in renal failure, and we assume that among the anions then present is citrate, or other substances akin to it in ability to complex calcium ions. Freeman and Chang (1950) showed that bilateral nephrectomy in dogs was followed by a large rise in the plasma-citrate level about 24 hours later, and that this was due to loss of ability of the renal parenchyma to remove citrate, since the rise did not occur after bilateral ligation of the ureters.

Patients with parathyroid adenomata (Cases 8, 9, 10, 11, and 12) did not show high levels of plasma complexed calcium except when the blood urea was above 100 mg. per 100 ml., as in Case 12, and in Case 9 (see above) on one occasion. This finding is inconsistent with the work of L'Heureux and Roth (1953), who found that in rats deprived of their thyroid and parathyroid glands the plasma-citrate levels were significantly lower than in normal rat plasma, and that when rats, whether normal or subjected to thyro-parathyroidectomy, were given parathormone an increase in plasma citrate followed. Our one patient with surgically induced hypoparathyroidism, F. C. (Case 13), was being treated with dihydrotachysterol, 6 mg. a day, when the plasma calcium fractions were first determined. The complexed fraction was then normal, only rising to a high level after she had been on this very large dose of dihydrotachysterol for about four weeks.

Shorr, Almy, Sloan, Taussky, and Toscani (1942) observed that when variations in urine calcium were induced in man and in post-menopausal women, whether normal, hypoparathyroid, or hyperparathyroid, there were parallel changes in urine citrate excretion. Chang and Freeman (1950) infused sodium citrate intravenously into female dogs, and found that a great increase in urine calcium then occurred. Chen and Neuman (1955) suggested that the urine calcium might be a function of a non-ionized complex of calcium

in the plasma. In four of our patients (Cases 13, 28, 29, and 44) there are data consistent with the view that urine calcium is controlled to a considerable extent by the plasma complexed calcium. In each of these cases figures are given of urine calcium excretion per day at two different levels of plasma complexed calcium. None of these patients had severe renal glomerular damage. In F. C. (Case 13) the urine calcium increased from 6 mg. to 253 mg. a day when the plasma complexed calcium values were 0·47 mg. and 1·0 mg. per 100 ml. respectively, the respective levels of plasma ionized calcium being 3·7 mg. and 4·6 mg. per 100 ml. The high concentration of urine calcium on the second occasion is unlikely to have been due to the increase in the ionized calcium fraction, since this fraction, though higher than before, was still subnormal, and the plasma total calcium remained below the usually accepted 'threshold'. It seems more likely to have been due to the abnormally high complexed fraction. In A. G. (Case 28) the urine calcium rose from 49 mg. a day when he was untreated to 398 mg. a day when he was treated with dihydrotachysterol, 3 mg. a day, while the plasma complexed calcium increased from 0·28 mg. to 1·26 mg. per 100 ml. and the plasma ionized calcium only from 6·2 mg. to 6·3 mg. per 100 ml. In M. E. (Case 29) the urine calcium increased from 4 mg. to 200 mg. a day, while the plasma complexed calcium increased from 0·4 mg. to 0·7 mg. per 100 ml. and the plasma ionized calcium remained at a slightly low level (5·8 mg. per 100 ml.). In G. P. (Case 44) the urine calcium level was high on both occasions when plasma calcium fractions were determined. Urine calcium excretions were 325 mg. and 404 mg. a day, while plasma complexed calcium values were 1·2 mg. and 1·1 mg. per 100 ml. respectively, and the respective levels of plasma ionized calcium were 6·2 mg. and 8·2 mg. per 100 ml. These figures suggest to us that the urine calcium may be a function of the plasma complexed calcium. If this is so, then in the condition of idiopathic hypercalcuria, characterized by a normal or diminished total plasma calcium, it may well be that a departure from normal may be observed in the complexed fraction.

Significant variations may easily occur in this fraction without causing significant changes in the total plasma calcium. We have data regarding three patients with idiopathic hypercalcuria (Cases 35, 36, and 37). In each case the values of plasma total and ionized calcium were normal, but the daily urine calcium was considerably increased (see Table), and the blood-urea normal. In no case was there evidence of renal tubular acidosis, osteoporosis, Paget's disease, or neoplasm. A high level of plasma complexed calcium was found in T. L. (Case 37), suggesting that an excess of complexed calcium was presented to the renal tubule, causing hypercalcuria by an 'overflow' mechanism. In K. S. and J. A. (Cases 35 and 36) the values of plasma complexed calcium were surprisingly low. This finding may have no significance, but it may be that in these two patients there was a renal tubular leak of the main calcium complex in the plasma. Clearly more work is required to elucidate this point. It is clear from the data regarding R. W. and E. P. (Cases 8 and 11), who had hyperparathyroidism, that their hypercalcuria was not due to an increase in the plasma

complexed calcium fraction. Two possible explanations of the hypercalcuria are apparent. Either a high level of plasma ionized calcium or a lowered threshold for the complexed fraction may have been responsible. It may well be that the urine calcium is a function mainly of the plasma complexed calcium, but also of the ionized fraction. It is hoped in the near future to make a further study of this point.

General Conclusions

It has proved both possible and practical to determine, in patients with a wide variety of diseases, with and without disturbance of acid-base balance, the ionized and complexed calcium levels in plasma ultrafiltrates. Determination of plasma ionized calcium takes only four man-hours once a working routine has been established, and gives valuable information in certain cases. The method has appeared to be reliable, since it gives the expected high results in cases of parathyroid adenoma and vitamin-D poisoning, and low results in cases of tetany following surgical removal of the parathyroids. Determination of the plasma ionized calcium concentration may be particularly rewarding in renal failure, when its level may be the only reliable criterion for deciding whether any accompanying hyperparathyroidism is of the primary or secondary type. In pathological conditions affecting plasma proteins or plasma pH, or both, it may be difficult or impossible to predict the correct value of the plasma ionized calcium by inferences based on measurements of plasma protein, and the only way to find the true value is to measure it. Knowledge of the true value may be necessary for the correct treatment of the osteodystrophy of renal failure or steatorrhoea. The determination of plasma complexed calcium may prove to be most important for a proper understanding of the renal excretion of calcium, and abnormalities of the plasma complexed calcium may be present in idiopathic hypercalcuria. Observation of plasma ionized calcium permits a new approach to be made to the problems of causation and prevention of tetany, and to the understanding of the abnormal calcium metabolism in many bone diseases.

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APPENDIX

Case Notes

Many of the patients, described here very briefly, have been investigated over long periods of time in the Metabolic Ward of University College Hospital.

It is hoped that some will be described in greater detail at a later date. All operations at University College Hospital (U.C.H.) in which parathyroid tumours were sought or found were performed by Mr. D. R. Davies.

Alkaline phosphatase is expressed in King–Armstrong units. The calcium, phosphorus, bicarbonate, and protein values in the plasma in each case will be found in the Table.

Parathyroid adenomata

Case 8, R. W. A woman, aged 53, had bilateral renal calculi of calcium oxalate and phosphate. There was no clinical or radiological evidence of bone disease. The plasma sodium, potassium, bicarbonate, and chloride were normal, and alkaline phosphatase 13 units. Urea clearance was 68 per cent.; the 24-hour urine calcium was 235 mg. A chief-cell parathyroid adenoma (220 mg.) was removed on 3.6.57, and three other normal parathyroids identified.

Case 9, G. W. A woman, aged 44. Bilateral renal calculi were discovered in 1949. In October 1956 the serum calcium was 11·4 mg. per 100 ml.; she was transferred to U.C.H. three months later. There was no clinical or radiological evidence of bone disease. The plasma sodium and potassium were normal, chloride 111 m-equiv. per litre, and alkaline phosphatase 10 units; the blood urea was 69 mg. per 100 ml.; the 24-hour urine calcium was 297 mg. A large chief-cell parathyroid adenoma was found in the mediastinum at a second exploratory operation on 3.4.57.

Case 10, E. C. A woman, aged 67. An 'osteoclastoma' of the shin was removed in May 1956. She was bed-ridden from general weakness and bone pain when admitted to U.C.H. in January 1957. There were five inches of trunk shortening. The plasma chloride was 106 m-equiv. per litre, sodium and potassium normal, alkaline phosphatase 102 units, and blood urea 96 mg. per 100 ml. Extensive changes characteristic of hyperparathyroidism were seen in X-rays of the bones. A large chief-cell parathyroid adenoma was removed on 13.3.57. Two days later a low-calcium tetany developed; a further day later she died from a myocardial infarct.

Case 11, E. P. A woman, aged 31. In February 1957, when she was investigated because of pain in the shoulder, extensive radiological signs of hyperparathyroidism were observed. The plasma calcium was 16·3 mg., phosphorus 1·4 mg. per 100 ml., and alkaline phosphatase 45 units. She was admitted to U.C.H. on 7.4.57. There were no skeletal deformities. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 50 units. Urea clearance was 51 per cent., and the 24-hour urine calcium 520 mg. Bone X-rays confirmed the previous findings. Despite three extensive explorations of the neck and mediastinum, no parathyroid adenoma has been found. All who have knowledge of her remain convinced that she has such an adenoma, and it is hoped to devise methods to determine its location.

Case 12, D. B. A woman, aged 63, had two parathyroid adenomata and sarcoidosis; the latter diagnosis was based on the finding of bilateral iridocyclitis in 1937, uveoparotitis and bilateral hilar lymphadenopathy of the lungs in 1942, and multiple small pruritic nodules since 1942, shown by skin biopsy in 1949 to be sarcoidosis. In 1957 she had increasing dyspnoea and failing vision. The plasma sodium and potassium were normal, cholesterol 486 mg. per 100 ml., and blood urea 117 mg. per 100 ml. The 24-hour urine calcium was 230 mg. X-rays showed old sarcoid cysts in the phalanges of her fingers. After calciferol 1,800 i.u. a day for 10 days the plasma calcium increased from 11·7 to 13·1 mg. per 100 ml.; with cortisone 150 mg. a day the level fell to

10·6 mg. per 100 ml. Autopsy revealed two chief-cell parathyroid adenomata, and changes in the hilar glands of the lungs consistent with healed tuberculosis or sarcoidosis.

Hypoparathyroidism

Case 13, F. C. A woman, aged 43. In November 1956 partial thyroidectomy for thyrotoxicosis was followed by a tetanic convulsion (plasma calcium 4·2 mg. per 100 ml.). She was given thyroid, 1½ gr. a day, with dihydrotachysterol 6 mg. a day from 30.12.56 for four weeks (then reduced to 2 mg. a day). From 6.1.57 to 23.1.57 a positive calcium balance of 700 mg. a day showed that she was recalcifying bones rendered deficient of calcium by long-standing thyrotoxicosis. The plasma calcium fractions were determined on 11.1.57 and 29.1.57.

Nephrotic syndrome

Case 14, D. H. A boy, aged 12, was found in 1952 to have proteinuria, a blood-pressure of 145/110, hepato-splenomegaly, and a low plasma-protein level of nephrotic pattern, with normal urea clearance. In April 1957 he was admitted to U.C.H. with headaches and swollen ankles. The blood-pressure was 170/140. The liver was palpable two to three fingers below the right costal margin, and a firm spleen was palpable two fingers below the left costal margin. The plasma sodium, potassium, and chloride were normal, cholesterol 645 mg. per 100 ml., and alkaline phosphatase 8·7 units. Urea clearance was 71 per cent. Intravenous pyelography showed symmetrically enlarged kidneys with increased cortical thickness and cortical calcification.

Case 15, L. W. A man, aged 41. In 1945 he had had headaches for one year, and swollen ankles for three months; the urine contained 1·3 per cent. protein, the plasma albumin was 2·5 per cent., and urea clearance 150 per cent. He was admitted to U.C.H. in 1946, 1950, 1951, 1953, and 1954 with various degrees of pitting oedema, and treated with a high-protein, low-salt diet, and katonium ion-exchange resin. He was readmitted in January 1957; the plasma sodium and potassium were normal. The plasma calcium fractions were determined when the patient was free of oedema, after prednisone 10 mg. a day for a week, and when the blood urea and electrolytes were normal. Katonium had been stopped.

Case 16, T. W. A boy, aged 13, with Ellis type 2 nephritis. In 1948 he developed generalized oedema, which lasted for 21 months. It recurred in 1953, becoming gross in January 1954. Admitted to U.C.H. April 1954, he had anasarca, with urine protein 1·6 to 2·0 per cent., plasma albumin 0·6 per cent., and globulin 3·7 per cent. The plasma calcium fractions were determined in May 1957, on his eighth admission to hospital and during katonium therapy. The plasma sodium, potassium, and chloride were normal, cholesterol 586 mg. per 100 ml., and alkaline phosphatase 8·5 units; urea clearance was 71 per cent. Renal biopsies showed changes characteristic of Ellis type 2 nephritis.

Case 17, L. C. A woman, aged 33, with amyloidosis and the nephrotic syndrome. From 1948 to 1950 she was subject to an evening temperature of 102° to 104° F. In February 1956 she had swelling of the face and ankles, serum albumin 1·7 per cent. and globulin 2·8 per cent., and urine protein 0·1 per cent. She was admitted to U.C.H. in January 1957; a firm spleen and liver were palpable two fingers below the costal margins; the haemoglobin was 46 per cent., plasma alkaline phosphatase 4·4 units, and cholesterol 207 mg. per 100 ml. The plasma calcium fractions were determined on 8.2.57. She died on

2.4.57 after a subarachnoid haemorrhage. Autopsy showed amyloid in the liver and kidneys.

Case 18, D. K. A man, aged 41, with Ellis type 2 nephritis and chronic renal failure. He had swelling of the legs and scrotum in 1954, and was admitted to U.C.H. The plasma albumin was 2·6 per cent., globulin 1·9 per cent., and cholesterol 372 mg. per 100 ml. Urea clearance was 34 per cent. and urine protein 0·1 per cent. He improved with low-salt and high-protein diet, but was readmitted with gross oedema in November 1955, when the blood urea was 81 mg. per 100 ml. He improved with ion-exchange resins, but was readmitted in June 1957 with acute pulmonary oedema and anasarca. The Chvostek and Trousseau signs were negative; haemoglobin 50 per cent., and blood-urea 240 mg. per 100 ml. The plasma sodium was 139 m-equiv. and potassium 6·4 m-equiv. per litre, and alkaline phosphatase 5·6 units. The plasma calcium fractions were determined on 21.6.57. He died on 13.9.57, and autopsy showed a granular 'horse-shoe' kidney with numerous hyalinized glomeruli. The parathyroids were about twice the normal size, but of normal microscopic appearance. Ion-exchange resins had not been given for several months when the calcium fractions were measured.

Chronic renal failure

Case 19, U. B. A woman, aged 33. In 1953 she had oedema and blurring of vision; she was hypertensive, with grade IV retinopathy and chronic bilateral pyelonephritis. The left kidney was removed, with no resultant fall in blood pressure. She was treated with pentolinium, then hydrallazine, then reserpine. In March 1957 the retinopathy was grade II–III, the blood-urea and plasma values as shown in the Table, plasma sodium and potassium normal, alkaline phosphatase 6·1 units, and magnesium 2·5 mg. per 100 ml.

Case 20, K. N. A boy aged 11, with Ellis type 1 nephritis and chronic renal failure. Haematuria had followed scarlet fever at five years, and occurred again after sore throats twice in the next three years. He was admitted to U.C.H. in March 1957. The blood-pressure was 140/100, haemoglobin 32 per cent., and urine protein 0·4 per cent. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 14 units. The plasma calcium fractions were determined before and after treatment with sodium bicarbonate 3 g. a day for 16 days, which caused alkalosis (see Table). He died on 2.6.57, and autopsy showed very small granular kidneys.

Case 21, A. W. A woman, aged 41, with chronic renal failure and secondary hyperparathyroidism. She had had thirst and polyuria since childhood. X-rays of the left shoulder after a minor injury in 1956 revealed gross changes due to hyperparathyroidism. She was admitted to U.C.H. in March 1957. The plasma sodium and potassium were normal, and alkaline phosphatase 91 units; fat-balance was normal. The plasma calcium fractions were determined before and after 21 days of calciferol therapy (2 mg. a day). This treatment changed the calcium balance from −12 to +1,182 mg. a day.

Case 22, A. C. A girl, aged 15, with incoordination of the bladder neck, chronic renal failure, rickets, and secondary hyperparathyroidism. In 1952 enuresis had followed an acute urinary infection; intravenous pyelography showed bilateral hydronephrosis, hydroureters, and a large bladder; the blood urea was 36 mg. per 100 ml. In 1953 part of the bladder wall and internal sphincter were resected. Recurrent urinary infections continued, and she was admitted to U.C.H. in March 1955. Her height was 58 inches. The blood-pressure was 160/110, and blood urea 84 mg. per 100 ml. The plasma calcium, phosphorus, sodium, potassium, and chloride were normal, and alkaline

phosphatase 32 units. She was readmitted in March 1957; her height was 59 inches, blood-pressure 150/110, and blood urea 123 mg. per 100 ml.; the plasma sodium and potassium were normal, and alkaline phosphatase 57 units. X-rays of the bones showed rickets with hyperparathyroidism.

Case 23, S. P. A girl, aged 18, suffering from renal failure with rickets and secondary hyperparathryoidism. She had had attacks of Ellis type 1 nephritis at two, five, seven, and 10 years. Growth ceased at 15 years. At 16 years she had pains in the legs and back on standing or walking, and at 17 she needed two sticks to stand. The blood urea was 90 mg. per 100 ml., the plasma calcium 8·3 mg., and phosphorus 5·5 mg. per 100 ml., and alkaline phosphatase 110 units. X-rays of the bones showed severe hyperparathyroidism without rickets or osteomalacia. She was treated, under close metabolic observation, with large doses of dihydrotachysterol, and made a spectacular recovery clinically, biochemically, and radiologically, the bones apparently returning to normal. The first determination of plasma calcium fractions was on 3.4.57, when she was well and free of symptoms, receiving dihydrotachysterol 0·125 mg. a day. Six weeks later the level of plasma ionized calcium was found to be low, and bone symptoms returned; on 5.6.57 the dose was increased to 0·25 mg. a day. The next determination of plasma calcium fractions was on 11.7.57, when she was clinically improved, but not yet free of symptoms.

Case 24, G. P. (to be published shortly by Professor C. E. Dent). A boy, aged 16, suffering from chronic renal failure with renal tubular abnormalities (Fanconi syndrome without cystinosis) and rickets. There had been life-long thirst. Knock-knees developed at 11 years, and became worse in the next two years until, when the patient was admitted to U.C.H. in June 1954, there was a gap of eight and three-quarter inches between the medial malleoli. The plasma calcium was 9·5 mg. and phosphorus 3·8 mg. per 100 ml., and alkaline phosphatase 61 units; the plasma sodium and potassium were normal, and bicarbonate 19 m-equiv. per litre. The blood urea was 48 mg. per 100 ml. The urine contained excess of protein, sugar, cystine, and many other amino acids. X-rays showed severe rickets at the wrists, knees, and ankles, with evidence of hyperparathyroidism in the hands, radii, ulnae, and pubic rami. He was treated with sodium bicarbonate 8 g. a day, and dihydrotachysterol up to 3 mg. a day. The medial femoral condyles were stapled. By April 1957, when the plasma calcium fractions were first determined, the inter-malleolar distance was one and a half inches, he was free of symptoms, and X-rays of the bones appeared normal. Dihydrotachysterol, 3 mg. a day, was continued until August 1957, when the plasma calcium fractions were again determined. He was then well and working full time.

Renal tubular osteomalacia (and rickets)

Case 25, V. C. (One of the four cases of a syndrome described by Dent and Harris (1956)). A woman of 24, with type 1 renal tubular osteomalacia. She had stopped growing, and developed severe pains in the back and groins, at 13½ years. Symptoms progressed in spite of vitamin-D therapy until 1953, when she could hardly walk, and was admitted to U.C.H. There was general bone tenderness; she was dwarfed, with four and a half inches of trunk shortening. The urine contained an excess of glycine. Urea clearance was 114 per cent. Fat-balance was normal. The plasma calcium was 9·1 mg. and phosphorus 1·7 mg. per 100 ml., and alkaline phosphatase 20 units. X-rays of the bones showed severe osteomalacia. There were normal metabolic responses to ammonium chloride. She was treated with dihydrotachysterol, 5 mg. a day, from September 1954. By May 1957, when the plasma calcium fractions were

determined, she was virtually free from pain and active; the bones had healed radiologically; the plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 24 units.

Case 26, M. F. A woman, aged 57, with type 1 renal tubular osteomalacia. Bow legs had resulted from rickets at 18 months. Growth stopped at 15 years, with the legs still bowed. Two children had died in infancy; she had one normal child, and one son who grew up to have bow legs and 'vitamin-D resistant rickets'. In 1936, after her fourth child was born, she developed low backache, and pain and stiffness in the thighs. She was admitted to U.C.H. in May 1957. Her height was 48 inches, and span 56 inches. There was a gap of six inches between the knees when the medial malleoli were touching. The plasma alkaline phosphatase was 19 units. The urine showed excess of glycine. X-rays showed a triradiate pelvis, 'cod-fish' vertebrae, and a Looser zone in each ulna.

Case 27, K. W. A girl, aged eight, with type 2 renal tubular rickets. She had developed rickets at two years, and her gait steadily deteriorated until 1955, when she was given vitamin D up to 45,000 i.u. daily. The rickets improved, but therapy was stopped in November 1955, and rickets recurred in the following June. A trace of sugar was then noted in the urine. She was admitted to U.C.H. in October 1956. Her height was 43¾ inches, and she had bow legs. The 24-hour urine glucose was 1·1 g. Urea clearance was 80 per cent. The plasma calcium was 9·8 mg. and phosphorus 2·4 mg. per 100 ml., alkaline phosphatase 27 units, and bicarbonate 24·2 m-equiv. per litre. X-rays showed rickets at the wrists and knees. Her positive calcium balance increased from 300 to 450 mg. a day on vitamin D_3 3 mg. a day. She was readmitted March 1957, receiving the same dose of vitamin D_3. The plasma alkaline phosphatase was 24 units, and other values as shown in the Table. X-rays showed healing of the rickets, with normal new growth of bone.

Case 28, A. G. A man, aged 39, with type 2 renal tubular osteomalacia. He had had increasing weakness and generalized bone pains from 1947 until he was admitted to U.C.H. in June 1957. He was aware of recent loss of several inches in height. He had a waddling gait, using two sticks. There was bone tenderness, and a loss of six inches in height above the pubis. The 24-hour urine contained glucose, 3·9 to 7 g., and there was an excess of glycine. Urea clearance was 129 per cent. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 48 units. The blood sugar was normal, and vitamin-A absorption normal. X-rays of the bones showed multiple Looser zones. The calcium balance was −100 mg. a day, with a low level of urine calcium. The plasma calcium fractions were determined before and after 49 days of dihydrotachysterol (2 mg. a day).

Steatorrhoea

Case 29, M. E. A woman, aged 51. She had had increasing weakness and 'stiffness', with generalized bone pains, from 1952 to 1956, when she was found to have multiple Looser zones, 79 per cent. fat-absorption, and megaloblastic anaemia. Overt steatorrhoea then developed. She was treated with a gluten-free diet, folic acid, calciferol 600,000 i.u. fortnightly, calcium gluconate, and intramuscular iron. In October 1956 she had spiral fractures of both tibiae. She was admitted to U.C.H. in November 1956. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 23 units. There was excess of glycine in the urine. Urea clearance was 130 per cent. Fat-absorption was 60 per cent. X-rays showed severe osteomalacia. The calcium balance, on a gluten-free diet and calciferol as shown above, was +130 mg. a day. The plasma calcium fractions were first determined when she had had calciferol 15 mg. a

day for 15 days, and when the calcium balance was +1·3 g. a day. They were determined again when she was readmitted, still receiving calciferol 15 mg. a day, in June 1957, when X-rays showed healing of the Looser zones and the calcium balance was +1 g. a day.

Case 30, F. A. A woman, aged 50, with steatorrhoea, myopathy, and a hiatus hernia. In 1926 she had had episodes of fever, abdominal pain, constipation, and diarrhoea. Laparotomy showed a tuberculous ovary, and many tubercles on the small intestine. Since then there had been intermittent diarrhoea, with occasional severe exacerbations, and since 1941 generalized muscular weakness, worse when the diarrhoea was worse. The history of hiatus hernia dated from 1944. She was admitted to U.C.H. in 1957. The tongue was smooth and red; she had a waddling gait, muscle hypotonia, and muscle weakness most marked proximally. The plasma sodium and potassium were normal, and alkaline phosphatase 9·4 units. Fat-absorption was 70 per cent. The bones were normal radiologically. An electromyogram was normal; the muscular weakness was unaffected by prostigmine. A trial of gluten-free diet did not help her.

Case 31, H. M. A woman, aged 60, with primary biliary cirrhosis and steatorrhoea. In 1951 she had jaundice, pyrexia, and abdominal pain, with hepatosplenomegaly. Liver biopsy showed multilobular fibrosis with bile-duct proliferation. She continued to have jaundice and frequent pale stools, and was admitted to U.C.H. in 1957 when backache and pains around the shoulders had developed. She had normal faecal and urine calcium, and was in calcium balance. Fat-absorption was 71 per cent. Bone X-rays showed only slight generalized rarefaction.

Osteoporosis

Case 32, I. M. A woman, aged 52, with idiopathic osteoporosis, recurrent vertebral and rib fractures, kyphosis, and reduced vital capacity. In 1952, at the menopause, she had noticed loss of height, and pains in the right leg, which were followed by a spontaneous fracture of the femoral neck. In 1953 she had many spontaneous fractures. She was admitted to U.C.H. in May 1957. Her height from crown to pubis was 28 inches and from pubis to heel 33 inches, and span 65½ inches. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 8·0 units. The blood urea was 18 mg. per 100 ml. Her vital capacity was 1,082 ml. The 24-hour 17-keto- and ketogenic steroids in the urine were 6·8 and 14·8 mg. respectively. X-rays showed severe osteoporosis of all bones, with many fractures.

Case 33, W. A. A man, aged 37, with idiopathic osteoporosis. (Described by Anderson (1954)). Plasma infusions were continued, and no further loss of height occurred. The plasma calcium fractions were determined four months after plasma infusion.

Case 34, E. E. A woman, aged 60, with senile osteoporosis. In 1953 spontaneous fracture occurred through a Looser zone in the right femur. The serum calcium was 9·2 mg. and phosphorus 3·8 mg. per 100 ml., alkaline phosphatase 9·4 units, and bicarbonate normal. The blood urea was 24 mg. per 100 ml. The 24-hour urine calcium was 272 and 396 mg. Fat-balance was normal. The fracture healed well. In 1954 she had a spontaneous fracture of the left femur. The plasma calcium, phosphorus, and phosphatase were again normal; there were high levels of urine and faecal calcium. The fracture again healed well. With stilboestral 2 mg. a day, and calciferol 1 mg. a day, the urine calcium fell to 115 mg. a day. The patient was admitted again in April 1957, still receiving this treatment. The plasma sodium, potassium, and chloride were

normal; other values are shown in the Table. Vitamin-A absorption was normal. Calcium balance was slightly positive, with normal faecal and urine calcium.

Idiopathic hypercalcuria

Case 35, K. S. A man, aged 36, with idiopathic hypercalcuria and renal stones. In 1953 a stone was removed from the left ureter after four attacks of left renal colic; the blood urea was 26 mg. per 100 ml.; *Staphylococcus albus* was present in the urine. He had left and right renal colic in 1954 and 1955 respectively. The plasma calcium, phosphorus, and bicarbonate were normal. The 24-hour urine calcium was 588 mg. and 669 mg. on a normal diet. Left partial nephrectomy was performed in March 1956. In August 1956 the urine calcium was 583 mg. a day. In January 1957 the urine was sterile; there were normal metabolic responses to ammonium chloride.

Case 36, J. A. A man, aged 40, with idiopathic hypercalcuria and renal stones. He had left renal colic four times between 1942 and 1956, when bilateral renal calculi were observed. The plasma calcium and phosphorus were normal, but the urine calcium was 350 to 400 mg. a day on a normal diet. The blood urea was 20 mg. per 100 ml., and plasma bicarbonate 23 m-equiv. per litre. With oral ammonium chloride the urine pH fell only to 5·3, but there was no reduction in urine calcium when he was given sodium bicarbonate 4 g. a day. The slight degree of renal tubular acidosis was therefore thought secondary to idiopathic hypercalcuria.

Case 37, T. L. A man, aged 34, with idiopathic hypercalcuria and nephrocalcinosis. He was given milk and alkali therapy for seven years up to 1956, when he had acute pyelonephritis in April and September. The plasma sodium, potassium, and chloride were normal, and alkaline phosphatase 7 units. Urea clearance was 63 per cent. There were normal metabolic responses to ammonium chloride. The urine calcium was 269 mg. a day. X-rays showed bilateral nephrocalcinosis.

Idiopathic tetany

Case 38, M. H. A boy, aged 16. Severe tetany, possibly following hyperventilation, was observed on his third day in hospital, where he was admitted for treatment of tonsillitis. Tetany was relieved by intravenous calcium gluconate. The Chvostek sign was repeatedly present in the following two weeks.

Case 39, R. B. A man, aged 47, who had had increasing stiffness of the hands, with paraesthesiae, for two years, and deterioration in handwriting. The Chvostek sign was always present, but the Trousseau sign absent.

Case 40, S. I. A woman, aged 42, who had had subtotal thyroidectomy in 1948. From 1952 to 1954 there was increasing flexor spasm of all the fingers, with paraesthesiae, worst on waking in the morning. In 1954 the Trousseau and Chvostek signs were positive. The plasma calcium, phosphorus, bicarbonate, and potassium were normal. In April 1957 the symptoms and signs were unchanged.

Case 41, S. W. A woman, aged 36. She had three attacks of nocturnal tetany in two months before her admission, but no attacks before this. The Trousseau sign was positive, but the Chvostek sign negative. The plasma sodium and chloride were normal, and alkaline phosphatase 6 units. Urea clearance was 100 per cent. Vitamin-A absorption was normal. X-rays of the bones were normal. The plasma calcium fractions were determined before giving ammonium chloride 5 g. a day for five days. This temporarily relieved the tetany, although the urine pH readily fell to 4·7.

Case 42, J. W. A woman, aged 27, who had gonadal agenesis with osteoporosis and tetany. (The patient of Geffen (1956).) She was readmitted to U.C.H. in March 1956 after oestrogen therapy. Severe tetany persisted, and the plasma calcium was 7·6 mg. per 100 ml., and potassium 3·1 m-equiv. per litre; other values were normal. With dihydrotachysterol, 3·75 mg. a day, the plasma calcium increased to 9·1 mg. per 100 ml., with relief of her tetany. The plasma calcium fractions were determined January 1957 after a 10-week period without dihydrotachysterol, and when the Trousseau and Chvostek signs were positive.

Case 43, L. R. A woman, aged 47, with vitamin-D deficient osteomalacia. (The case of Dent (1957*a*).) She was treated with dihydrotachysterol, 2 mg. a day, for six months, then 0·25 mg. a day for two and a half months, then with calciferol, 0·25 mg. a day, for three months. Then, in April 1957, there was no bone tenderness; the calcium balance was +100 mg. a day, with 250 mg. a day in the urine. The plasma alkaline phosphatase was 7 units.

Case 44, G. P. A girl, aged nine, who had hypo-hyperparathyroidism without steatorrhoea, as mentioned by Dent (1957*b*). In 1955 she had a waddling gait, and pain in the left knee. Leg-extension was applied for 10 weeks, then vitamin D (in cod-liver oil), 7,800 i.u. a day, was given for a month. After this she could walk with crutches, and her gait improved further on a reducing diet. In March 1956 she had her one and only attack of tetany; this lasted an hour. In April 1956 she was admitted to U.C.H. She had no food idiosyncrasies. She was tall and plump. The Chvostek sign was positive twice. The plasma sodium and potassium were normal, calcium 7·6 mg. and phosphorus 8·8 mg. per 100 ml., and bicarbonate 17·8 m-equiv. per litre; the plasma albumin was 4·4 g. and globulin 2·7 g. per 100 ml., and alkaline phosphatase 40 units. Urea clearance was 66 per cent. Fat-absorption was 97 per cent. X-rays of the bones showed severe hyperparathyroidism, no rachitic changes, and bilateral coxa vara. She was treated with vitamin D_3, 5 mg. a day for four months, then 3 mg. a day for six months. The plasma calcium fractions were first determined in March 1957, when she was free from pain and the bones were almost healed radiologically; the plasma alkaline phosphatase was 22 units. The blood urea was 42 mg. per 100 ml. The 24-hour urine calcium was 325 mg. The dose of vitamin D_3 was maintained until the second determination of plasma calcium fractions in July 1957; the plasma alkaline phosphatase was then 14 units, and the blood urea 78 mg. per 100 ml.

Summary

1. Results are reported of 66 determinations of plasma ionized, complexed, and total calcium in seven normal men and in 37 patients with a wide variety of pathological conditions affecting calcium metabolism.

2. In primary hyperparathyroidism the level of plasma ionized calcium is high, whereas in secondary hyperparathyroidism it is normal or low. There is some evidence that in primary hyperparathyroidism the binding of calcium by plasma proteins is lower than might be expected. This explains why some patients with parathyroid adenomata have normal levels of total plasma calcium.

3. The plasma ionized calcium is found to be diminished in hypoparathyroidism in accordance with expectation.

4. In two out of three cases of uncomplicated nephrotic syndrome the ionized

calcium was normal, the diminished total calcium in the plasma being entirely due to a decrease of the protein-bound fraction associated with a low level of plasma albumin. In the third case the ionized fraction was diminished.

5. In untreated renal glomerular osteodystrophy the level of plasma ionized calcium is low or normal. Its value is a good guide for controlling the dose of vitamin D required to heal the bone disease.

6. In two untreated cases of renal tubular rickets and osteomalacia (types 1 and 2) the plasma ionized calcium was normal. There was no 'tendency' to a low level of plasma ionized calcium, as implied by other workers.

7. In steatorrhoea the level of plasma ionized calcium was low in patients in whom it was suspected that osteomalacia was developing.

8. Overbreathing in normal subjects is followed by a decrease in the plasma ionized calcium. Nearly half of this decrease is due to an increase in the plasma complexed calcium, the remainder being due to an increase in calcium bound to plasma protein.

9. Four patients have been investigated who had chronic signs of tetany or pre-tetany in the presence of normal plasma electrolytes. All had normal plasma calcium fractions, and showed a normal decrease in the ionized fraction after overbreathing.

10. In cases of renal failure with hypocalcaemia, but without tetany, the absence of tetany is not due to acidosis alone. Protection must sometimes be due mainly to some other factor, which may be an increase of plasma magnesium.

11. In hypoparathyroidism tetany occurs when the plasma ionized calcium falls below a critical level of approximately 4·3 mg. per 100 ml., provided that there are no other complicating factors.

12. The level of plasma complexed calcium is high in alkalosis from overbreathing, and in renal failure when the blood-urea exceeds approximately 100 mg. per 100 ml. It is normal, however, in primary hyperparathyroidism uncomplicated by severe renal failure.

13. In the absence of marked renal glomerular damage the urine calcium has appeared to be related closely to the plasma complexed calcium, where clear evidence has been available. There is some evidence that there are two immediate causes of the high level of urine calcium in idiopathic hypercalcuria. Either there is an excess of complexed calcium in the plasma, or else there is a diminished renal tubular reabsorption of a complex of calcium.

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THE NEPHROTIC SYNDROME[1]

A Study of Renal Biopsies in 20 Adult Patients

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With Plates 49 to 51

The nephrotic syndrome is well recognized clinically in its typical form with the following characteristic features: (1) Generalized oedema; (2) Proteinuria; (3) Lowered concentration of plasma albumin; (4) Raised concentration of plasma cholesterol.

This clinical picture may occur with different pathological processes in the kidney, of which the best known are glomerulonephritis (Ellis type 2; membranous or lobular glomerulonephritis), amyloidosis, diabetic glomerulosclerosis, renal vein thrombosis, and systemic lupus erythematosus. It is, further, generally accepted that the nephrotic syndrome may occur with or without other complicating features, in particular hypertension or nitrogen retention, or as part of, or as a complication of some other illness. The nephrotic syndrome may have remissions and relapses, and in some instances a patient may recover completely without any apparent residue of renal damage. With this great variation in clinical presentation, it must be of the greatest importance to establish as nearly as possible a precise diagnosis if a valid prognosis is to be made, or if any therapeutic measures are to be assessed.

In the present series of 20 adult patients renal biopsy has been performed in each case, and repeated at an interval of some months in four of them. The main emphasis is given to an analysis of the histological findings. On this basis the patients have been separated into several groups. With the rather short follow-up period, amounting to two years at most, there is, up to the present, suggestive evidence that renal biopsies may be of prognostic value and should enable the clinician to know whether the patient has a type 2 glomerulonephritis or some other lesion. Inevitably, then, any apparently successful treatment must be set against the known renal histology. Although it is generally held that the severity of proteinuria can be paralleled by the amount of involvement of the glomerular basement membrane, and indeed may be dependent on it, several cases in the present series had a florid nephrotic syndrome without such glomerular changes. The criteria for including patients in the present study have been difficult to decide. If only those patients who showed all four features, namely,

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proteinuria, lowered concentration of plasma albumin, increased plasma cholesterol, and generalized oedema, were to be included, then four of the eight patients with histological changes typical of type 2 glomerulonephritis might have been excluded. The only constant finding in the patients has been a heavy proteinuria and decreased plasma albumin. Those patients in the series who had no marked oedema had either a typical type 2 histological picture or a considerable increase of the plasma cholesterol, as well as proteinuria and changes in the plasma proteins.

Patients Investigated

Those included in the study are a consecutive series of patients with the nephrotic syndrome seen in a two-year period. In the first nephrotic patient in whom a renal biopsy was attempted no renal tissue was obtained, but subsequent biopsies were all successful. Table I sets out the main original and follow-up findings, with the known duration of proteinuria in relation to the time of renal biopsy.

Case Reports

The main chemical investigations are given in Table I.

Patient 1, a woman aged 42. Since her third pregnancy in 1949 she has had chronic varicose ulceration of the left leg. During this third pregnancy her blood-pressure is thought to have been raised, and swelling of left leg occurred during the third trimester. A live child was born, but with cyanotic congenital heart disease. An apparently normal pregnancy occurred in 1952. In December 1955 the patient caught a cold, felt very unwell, vomited, and was in bed for three weeks with a febrile episode. The left leg was hot and swollen at this time. In May 1956 she was admitted on account of the varicose ulcer on the left leg; there was considerable indurated swelling of the left leg, and some oedema of the right leg; the blood-pressure was 180/105, and the urine contained protein. She was subsequently readmitted under medical care in October 1956, having developed generalized oedema during the previous months.

On examination there was fairly gross generalized oedema. Venous pressure was not raised. The blood-pressure was 210/110. The fundi were normal. The left ventricle was clinically enlarged. The lower half of the left leg showed a brawny indurated oedema, with a fine pustular rash on its central area. Renal biopsy was performed on 20.11.56. She was treated with a salt-free diet containing 100 g. protein, and has remained fairly well as an out-patient, able to do light housework.

Conclusion. Chronic type 2 glomerulonephritis.

Patient 2, a youth aged 15, admitted to hospital 25.5.56. There was an indefinite history of earache two months previously, associated with a cold, lasting one week. His mother thought his face was slightly puffy and the urine dark. Some days later an episode of diarrhoea and vomiting occurred, with some discharge from the eyes, and he was treated with penicillin. His face was slightly puffy. The illness settled in the course of a week. In the middle of April he had a febrile episode lasting four days, during which his face was again puffy and the urine

dark. Ten days before admission to hospital he noticed swelling of the ankles in the evenings; he was very thirsty, and got up once a night to pass urine. The swelling gradually increased until it was present all day, reaching up to the knees; the patient's mother again thought his face was puffy.

On admission he looked healthy; there was slight oedema of the feet and legs, and a trace over the sacrum. The blood-pressure was 160/90; the fundi were normal. There were no other abnormal physical signs. A heavy proteinuria was found, and numerous casts, red cells, and white cells in the centrifuged deposit. The blood urea was 35 mg. per 100 ml. At the end of a week the oedema had almost disappeared, the blood-pressure was 135/50, and fairly heavy proteinuria continued. By 20.6.56 there was no oedema, the blood-pressure was 155/80, and the patient appeared to be recovering and started getting up. On 16.7.56 slight pyrexia developed, with a slight return of oedema. The blood-pressure was 165/90, blood-urea 75 mg. per 100 ml., and total plasma protein 4·2 g. per 100 ml. On 25.7.56 the antistreptolysin-0 titre was 100 units per ml. On 23.7.56 he was transferred to St. Mary's Hospital. Examination showed no oedema, blood-pressure 155/65, venous pressure not raised, and a heavy proteinuria, ranging from 3 g. to 12 g. in 24 hours. The plasma electrolytes were normal. The results of other investigations are shown in Table I. The first renal biopsy was on 31.7.56; the second was on 2.4.57. Apart from a quite transitory and moderate oedema, the patient showed every feature of the nephrotic syndrome, and the renal biopsies showed a progressive type 2 lesion. Treatment with prednisone was started on 9.8.56 and continued for the remainder of the follow-up period. Heavy proteinuria has continued, and progressive nitrogen retention developed, although the patient continued to live an almost normal schoolboy existence, apart from not playing games. One year after the onset of the illness, with a blood-urea concentration exceeding 100 mg. per 100 ml., the boy successfully took an examination for the A level General Certificate of Education.

Conclusion. A rapidly progressing type 2 nephritis.

Patient 3, a man aged 66, first seen as an out-patient on 6.9.56. Ten days previously he had noticed swelling of the right ankle, but otherwise felt well; there had been no preceding illness. During the subsequent nine days he developed a rapidly progressing generalized oedema.

On examination there was gross oedema up to the waist. Venous pressure was not raised. The blood-pressure was 220/104; the fundi were normal. The urine contained large quantities of protein. He was admitted immediately to hospital, and given a low-salt diet containing 60 g. of protein, and during the first five days he had a massive diuresis and lost all but a trace of oedema. The first renal biopsy was performed on 18.9.56, no more than three weeks after the first onset of oedema. He was discharged at the beginning of October, but was readmitted on 27.11.56, having again developed gross oedema, and a second renal biopsy was performed on 22.1.57. Since his second admission, in spite of treatment with prednisone, there has been no remission, with variable oedema, maintained hypertension, and some increase in blood-urea.

Conclusion. A rapidly progressing type 2 nephritis.

Patient 4, a man aged 41, was admitted to hospital in May 1957, with a seven-year history of continual diarrhoea; there were as many as 12 stools a day in the month before admission. He had never remained in bed, and had continued work, but during the last two years had lost 21 lb. in weight. In April 1957 proteinuria was discovered as an incidental finding. Renal biopsy was performed on 20.5.57. A first-stage colectomy and removal of the terminal ileum was performed on 22.5.57. A second-stage operation was performed on 3.9.57; on this

TABLE I

Main Biochemical and Clinical Data

Patient number	*Sex and age (years)*	*Date of tabulated investigations*	*Proteinuria (g./24 hrs. or 0 to ++++)*	*Plasma proteins (g./100 ml.)*		*Oedema (0 to ++++)*	*Serum cholesterol (mg./100 ml.)*	*Blood-pressure (mm. Hg.)*	*Blood urea (mg./100 ml.)*	*Known duration of proteinuria before biopsy*	*Dates of renal biopsy*	*Prednisone or cortisone*	*Brief clinical details when last seen*
				Total	*Albumin*								
1	F 42	Nov. 1956	++++	4·6	2·5	++	340	160/100	47–75	2 months	20.11.56	No	Sept. 1957. Doing housework. Oedema ++. Blood-pressure 200/108. Proteinuria ++. Blood urea 50 mg., plasma albumin 1·8 g./100 ml.
2	M 15	July 1956	++++	4·5	1·5	+ to 0	430	160/80	42	2 months	First 31.7.56	Yes	Oct. 1957. Beginning to feel unwell, and off school. No oedema. Blood-pressure 200/100. Proteinuria +++. Blood urea 120 mg. plasma albumin 3·2 g./100 ml.
		April 1957	17·0	6·4	1·9	0	570	180/85	230		Second 2.4.57		
3	M 66	Sept. 1956	8–15	4·5	2·9	++++	285	120/70 to 160/90	52	12 days	First 18.9.56	Yes	Sept. 1957. Still off work. Oedema +++. Blood-pressure 185/100. Proteinuria +++. Blood urea 70 mg./100 ml.
		Jan. 1957	8–15	4·0	1·8	+++	380	160/90 to 170/100	50		Second 22.1.57		
4	M 41	May 1957	++++	5·4	1·8	0		110/80	22	6 weeks	20.5.57	No	Sept. 1957. Well. In hospital for 2nd stage colectomy. No oedema. Proteinuria 3 g./24 hrs.
5	M 44	Dec. 1956	++++	3·1	1·0	++++	340	200/120	45	3 years	10.1.57	Yes	Sept. 1957. Working; feeling well. Minimal oedema. Blood-pressure 180/110. Proteinuria ++.
6	F 23	Jan. 1957	+++	6·5	3·8	++	340	200/130 to 220/160	128–140	3 months	4.6.57	No	Oct. 1957. Doing light housework. Blood-pressure 186/108. No oedema. Proteinuria +++. Blood urea 140 mg., plasma albumin 3·2 g./100 ml.
		June 1957	+++	6·4	3·4	0							
7	M 35	June 1957	++++	5·8	2·5	+ to 0	296	160/88	97	5 weeks	22.7.57	No	Oct. 1957. Back to light work 2 weeks. No oedema. Blood-pressure 142/82. Anaemic. Proteinuria ++. Blood urea 69 mg., plasma albumin 3·3 g./100 ml.
8	M 31	Sept. 1957	5–7	5·3	2·0	0	334	120/70	36	6 months	10.9.57	No	Oct. 1957. Full work. No oedema. Blood-pressure 120/70. Proteinuria +++. Only one month follow-up
9	M 71	Nov. 1955	12·0	3·8 (Esbach)	1·0	++++	425	190/105	60	2 months	13.12.55	Yes	Sept. 1957. Complete remission. No proteinuria or significant oedema since April 1956

10	M 60	July 1957	5–8·0	4·0	1·3	+++	250	155/95	40	6 weeks	23.7.57	No	June 1957. (By letter.) Very well; full activity. No oedema. Proteinuria 1 g./24 hrs. Blood-pressure 160/100. Blood urea 32 mg., plasma albumin 3·4 g./100 ml.
11	M 53	Nov. 1956	15	4·8	2·1	+	395	160/90	56	8 months	8.11.56	Yes	Aug. 1957. Only one month follow-up. No oedema. Proteinuria ++
12	F 55	April 1957	2–12·0	5·5	2·0	++	339	170/90	60	18 months	7.5.57	No	Aug. 1957. Feeling better. Minimal oedema. Venous collaterals more prominent. Proteinuria ++. Blood urea 55 mg., plasma albumin 2·5 g./100 ml.
13	M 53	May 1957	7–35	3·4	2·0	++++	540	180/110	40–128	6 months	17.6.57	Yes	Oct. 1957. Feeling well. Oedema ++. Blood-pressure 140/105. Proteinuria ++. Blood urea 30 mg./100 ml.
14	M 55	April 1956	+++	4·6	1·5	+	350	150/90	55	9 months	4.5.56	No	Refused to attend hospital: 'too busy gardening'
15	M 55	Dec. 1956	6–10	3·9	1·5	++	280	140/80	50–98	3 months	5.2.57	Yes	April 1957. Feeling well. No oedema. Blood-pressure 140/80. Proteinuria 4 g./24 hrs. Blood urea 42 mg., plasma albumin 3·4 g./100 ml.
16	M 19	April 1957	8·7	5·0	1·8	+ to 0	433	150/86	26	3 months	14.5.57	Yes	Sept. 1957. Very well. Full work. No oedema. Blood-pressure 120/80. Proteinuria ++. Blood urea 32 mg., plasma albumin 4·1 g./100 ml.
17	M 55	April 1957	+++	7·5	2·9	++++	148	180/110	65	6 weeks	28.5.57	No	Aug. 1957. Well. No oedema. Blood-pressure 135/80. Proteinuria, faint trace. Blood urea 42 mg., plasma albumin 4·4 g./100 ml.
18	M 51	Aug. 1956	++++	4·0	1·3	++++	550	160/85	75	3 weeks	First 21.8.56	Yes	Sept. 1957. Complete remission. No oedema. Blood-pressure 150/90. No proteinuria. Blood urea 46 mg., plasma albumin 3·2 g./100 ml.
		Feb. 1957	0	6·0	1·5	trace	300	130/80	40		Second 22.2.57		
19	F 81	Sept. 1955	++++	4·9	1·6	++++	345	145/70	60	5 months	6.1.56	Yes	Sept. 1957. (By letter only.) Complete remission. No oedema. No proteinuria
		Dec. 1955	2–4	4·0	1·0	++++	285	140/70	51				
20	F 19	Dec. 1956	5–16	4·0	2·0	++	190	135/70	10	4 months	First 2.4.57	Yes	Sept. 57. Complete remission. No oedema. Blood-pressure 104/72. No proteinuria. Blood urea 30 mg./100 ml.
		April 1957	5	5·3	2·0	+	346	135/60	21		Second 24.9.57		
		Sept. 1957	0	5·9	3·5	0	168	104/72	30				

admission a single 24-hour collection showed a loss of less than 3 g. of protein in the 24 hours. At no time had there been any oedema.

Conclusion. An early type 2 lesion. Follow-up too short for any further assessment.

Patient 5, a man aged 44. In 1953 he had had an episode of facial swelling, which gradually increased for two weeks and became very marked; there was no swelling of the legs. Protein was found in the urine. He was in bed for two months in hospital, and returned to work two weeks after his discharge. He remained perfectly well until September 1956, when he developed generalized oedema, which gradually increased for four months. He continued working, but at the end of this period felt hot and shivery, and two days later was admitted to hospital with a temperature of 104° F, generalized gross oedema, a blood-pressure of 200/120, and proteinuria. Pneumonia was suspected, and he was treated with terramycin for six days. A renal biopsy was performed on 10.1.57, when the oedema had almost disappeared but proteinuria persisted. He was discharged by the end of January, and when seen as an out-patient on 18.2.57 he was well and at work; there was no oedema, the blood-pressure was 180/110, blood urea 37 mg. per 100 ml., and total plasma proteins 7·5 g. per 100 ml., with albumin 3·2 g. per 100 ml. He remained well and at full work until last seen on 9.9.57, when there was minimal oedema, the blood-pressure was unchanged, and the urinary protein concentration 140 mg. per 100 ml.

Conclusion. Type 2 nephritis.

Patient 6, a woman aged 23, was first admitted to hospital in March 1957, with a vague history of swelling of the legs over the last year and a half. There had been no dysuria, but nocturia once or twice a night for a year or more. On admission to hospital she had fairly gross generalized oedema. The blood-pressure was 220/130. There was heavy proteinuria, and generalized oedema which gradually settled and had disappeared by the time of renal biopsy on 4.6.57. When last seen in September 1957 she had a faint brownish skin pigmentation, and some fullness of the face, but no other oedema. The left ventricle appeared enlarged clinically. The patient was up and about at home, doing light housework. No steroid had been given, but hypotensive drugs were used to control the blood-pressure.

Conclusion. Severe chronic type 2 nephritis.

Patient 7, a man aged 35. In the middle of May 1957 he developed a vesicular rash on the volar surfaces of the wrists, diffusely on the legs, and very sparsely on the face. The rash was very irritating, and the vesicles increased in size, but at the end of two weeks it had largely resolved, leaving only patches of brownish pigmentation. At this time a red exfoliative dermatitis developed on his feet and ankles, and shortly afterwards there was moderate swelling of both legs up to the knees, and transient painful swelling of the wrists and hands.

He was admitted to hospital on 15.6.57. There was an exfoliative dermatitis of both ankles and feet, with some oedema up to the knees. The blood-pressure was 160/88. Venous pressure was observed just above the sternal angle, there was no tachycardia, and the heart was normal. He was treated with rest in bed and a low-salt diet, and subsequently with a high protein intake. The blood-urea level gradually fell from 97 mg. to 60 mg. per 100 ml. by 29.7.57. The skin rash settled rapidly, although some exfoliation on the soles of the feet was still present four weeks later. The oedema had settled in a few days. When last seen in

October 1957 he was about to return to work; there was no oedema, fairly heavy proteinuria, and the blood urea was still above normal.

Conclusion. An early type 2 nephritis.

Patient 8, a man aged 31. In March 1957 he attended as an out-patient, with a four months' history of intermittent epigastric pain coming on about two hours after food and relieved by alkalis. During this time he had had three to four normally formed motions per day, no diarrhoea, and no melaena. A barium meal showed the presence of an ulcer crater on the lesser curve of the stomach. On examination he looked healthy. There was no oedema. The blood-pressure was 130/70; the fundi were normal. There was minimal epigastric tenderness. A fairly heavy proteinuria was a chance finding. There was no previous history of renal disease. He was admitted to hospital in September 1957 for investigation of the persistent proteinuria. On examination there were no abnormal physical signs. The epigastric tenderness was no longer present. Renal biopsy was performed on 10.9.57.

The chance finding of proteinuria led to renal investigations. There had never been any other relevant signs or symptoms of renal disease. No follow-up has been possible.

Conclusion. Early type 2 nephritis.

Patient 9, a man aged 71. In July 1954 he had been in hospital with a cardiac infarction. After his recovery and discharge from hospital he received weekly injections of 2 ml. of mercaptomerin from November 1954, remaining well apart from minor anginal pain. In October 1955 he developed swelling of the ankles, which gradually progressed until there was gross oedema of the lower half of the body. On 27.10.55 proteinuria was first found, and mercaptomerin was stopped. He was admitted with gross oedema on 16.11.55. Venous pressure was up to the sternal angle. The blood-pressure was 190/105; the fundi were normal. There was heavy proteinuria. Treatment with oral cortisone was started on 10.12.55; a diuresis started on 26.12.55. By the time of his discharge on 14.1.56 only minimal oedema was present. Small doses of oral cortisone were continued until March 1957. By February the urine contained no protein, although there was still minimal oedema. When last seen in September 1957 he was surprisingly well for his age, although he had some anginal symptoms and shortness of breath on moderate effort. There was minimal oedema of the ankles, the venous pressure was not raised, the blood-pressure was 170/96, and there was no proteinuria.

Conclusion. A transient nephrotic syndrome, with apparently complete remission. The presumed cause of the nephrotic syndrome was a mercury intoxication.

Patient 10, a man aged 60, had suffered for many years from bronchitis. From 1955 he had persistent shortness of breath on exertion. In June 1956 mersalyl injections were started, in doses of 2 ml. two or three times weekly. In January 1957, for the first time, he developed oedema of the ankles. During the four months preceding his admission to hospital on 20.6.57 the oedema gradually increased. On examination he was slightly dyspnoeic. There was marked oedema of the legs, and moderate oedema over the sacrum. Venous pressure was 2 cm. above the sternal angle. The blood-pressure was 155/95. Scattered râles and expiratory rhonchi were heard in the lungs. He had several rotten tooth stumps. There was bilateral webbing of the ring and index fingers. He was treated with a high-protein diet, aminometradine, and katonium resin. Renal

biopsy was performed on 23.7.57. When seen as an out-patient on 28.8.57 he was feeling well; there was no oedema, but still a large quantity of protein in the urine.

Conclusion. Nephrotic syndrome, the presumed cause being mercury intoxication.

Patient 11, a man aged 53. In March 1956 his ankles began to swell in the evenings; there was no dyspnoea, and no symptoms relating to micturition. Shortly afterwards he was seen by his doctor; his blood-pressure was 150/90, and heavy proteinuria was found. A routine medical examination in 1954 had revealed no proteinuria. In October 1956 the patient came to this country for a medical opinion. On examination he appeared healthy. There was slight oedema of the ankles; the venous pressure was not raised. The blood-pressure was 160/90. An incidental finding was a large single cyst on the lower pole of the left kidney, which was excised. Renal biopsy was performed on 8.11.56. He was given long-term treatment with prednisone. Follow-up was by letter, as he lived in Turkey. Eight months after renal biopsy the proteinuria was only a little over 1 g. per 24 hours. The blood urea was normal, but there was little change in the plasma proteins or cholesterol. He continued at full work after his discharge.

Conclusion. An incomplete nephrotic syndrome, with histological changes characteristic of benign nephrosclerosis.

Patient 12, a woman aged 55. In December 1955 she had had fairly severe backache, with fever up to 104° F. Two months later she developed oedema of the legs, and was in hospital for five weeks. She was treated with a salt-free diet without any obvious improvement. After returning home she was up only for a few hours in the morning, the oedema remaining unchanged. In April 1957 she was admitted to another hospital. There was moderate oedema of the legs and sacrum. The blood-pressure was 170/90. Distended tortuous veins were present on the abdomen and back, becoming filled rapidly when the patient sat up. Venous pressure was up to the sternal angle. There was no ascites. The blood urea was 60 mg. per 100 ml. Renal biopsy was performed on 7.5.57. The patient was subsequently treated with a high-protein, low-salt diet, and long-term anticoagulants. When seen on 10.9.57 she was feeling better. There was minimal oedema. The venous collaterals on the trunk had become more prominent. The blood urea was 55 mg. per 100 ml., and plasma proteins 6·8 g. per 100 ml., with albumin 2·5 g. per 100 ml.

Conclusion. Nephrotic syndrome following inferior venacaval thrombosis.

Patient 13, a man aged 53, had begun in January 1957 to have pain on inspiration in the left side of the chest, with dyspnoea. At the same time he developed swelling of the ankles. X-rays of the chest showed no abnormality. He remained off work for six weeks, and the oedema gradually subsided. Two weeks after he had resumed work in March 1957 the oedema returned, and gradually increased until his admission on 28.4.57. On the day of admission there was an episode of aphasia and weakness of the right arm, lasting two hours.

On examination there was gross oedema to the waist. Venous pressure was not raised. The blood-pressure was 180/130. Some residual weakness was noted in the right hand. During the next few weeks several minor episodes of transient limb weakness occurred; the most obvious of these, five days after admission, consisted of weakness of the whole left side, with residual weakness of the left arm lasting about two weeks. Dietetic treatment was given, and prednisone was

started on 12.5.57. The blood urea, which on admission had been 60 mg. per 100 ml. and had subsequently fallen to 40 mg. per 100 ml., rose to 128 mg. per 100 ml. on 21.5.57, but had fallen to 40 mg. per 100 ml. by 10.6.57. Renal biopsy was performed on 17.6.57. Subsequently anticoagulant therapy was begun; a maintenance dose of phenylindanedione was given (50 mg. twice daily), and the patient was discharged with moderate oedema on 24.7.57. He was last seen as an out-patient on 17.10.57, when the oedema was unchanged and there was still clumsiness of the left arm. The blood urea was 30 mg. per 100 ml.

Conclusion. Nephrotic syndrome, the presumed cause being renal vein thrombosis.

Patient 14, a man aged 55. In May 1955 he had developed swelling of the legs, which persisted until his admission to hospital for four weeks in August and September 1955. On admission there was gross oedema of the legs. The blood-pressure was 135/85, there was moderately heavy proteinuria, and the blood urea was 26 mg. per 100 ml. He was treated with mersalyl and a high-protein diet. On discharge there was very little oedema. He remained well except for peculiar cramp-like pains in the legs which occurred intermittently. From February to April 1956 he experienced recurrent flitting pains, lasting a few days at a time, in most joints. On 2.4.56 he developed fever, with severe painful paraesthesiae of the lower leg, and on 5.4.56 was admitted to hospital. On examination there was slight oedema of the legs. The blood-pressure was 150/90. Venous pressure was not raised. There was reddening and swelling of the left wrist. Renal biopsy was performed on 4.5.56. In view of a nephrotic syndrome associated with recurrent joint pains, and swelling observed in one joint, the diagnosis of systemic lupus erythematosus was considered a possibility. The patient was unfortunately unwilling to attend hospital for any follow-up.

Conclusion. Chronic nephrotic syndrome. Possibly systemic lupus erythematosus.

Patient 15, a man aged 55. On 3.11.56 he ran an iron filing into his right thumb. On 10.11.56 the thumb had become very swollen and painful; he attended hospital, where the thumb was incised under a general anaesthetic in the Casualty Department. On 13.11.56 his left knee became hot, swollen, and tender, and the same evening he developed aching pains in the elbows and ankles. On 14.11.56 a rash appeared from the ankles to the knees, with oedema up to the knees. The rash consisted of circular erythematous areas with a central purpuric spot. On 15.11.56 he was admitted to hospital. He appeared ill; the blood-pressure was 140/80, venous pressure was normal, and an erythematous and purpuric rash was present as described above. There was considerable oedema of both legs, the left ankle joint was hot and swollen, and there was slight generalized enlargement of the lymph-glands. The urine contained large quantities of protein, with moderate numbers of red cells and casts. During the next few days the oedema increased slightly, extending on to the trunk. Cortisone, 25 mg. four times a day, was given from 16.11.56 to 3.12.56; prednisone, 5 mg. thrice daily, was given from 31.12.56, the dose being reduced progressively and stopped one week later. Renal biopsy was performed on 5.2.57. When seen as an out-patient on 5.3.57 he was feeling well. The blood-pressure was 180/100, falling to 140/80 after resting. There was no oedema. The urine still contained large quantities of protein, and the erythrocyte sedimentation rate was 107 mm. in the first hour. He was seen again on 2.4.57, when there was again no oedema; the blood-pressure was 140/80.

Conclusion: Nephrotic syndrome with focal glomerulonephritis complicating a Schönlein–Henoch syndrome.

Patient 16, a man aged 19. On 3.2.57, while he was driving a motor bicycle in a hail-storm, his face suddenly swelled up quite grossly; the swelling diminished after an hour, but remained for five days. The day after this episode he was seen by his own doctor, who found proteinuria. There was no other relevant history, although he had felt generally unwell during the preceding month. He had no oedema of the ankles, but had apparently gained some 9 lb. in weight by the time he was admitted to hospital on 8.2.57.

On examination there was no obvious oedema. The blood-pressure was 180/90. There was heavy proteinuria, with red cells and casts in the sediment. During the following weeks in hospital several peculiar attacks occurred, consisting of a severe frontal headache and transient blurring of vision, followed by vomiting; subsequently considerable puffiness of the face occurred, lasting about 20 minutes. On one occasion the facial oedema was associated with a local profuse purpuric eruption. He was treated with a 10-day course of cortisone, which resulted in a net loss of 4 lb. in body-weight; proteinuria disappeared for three days, but then again became heavy. He felt generally well, and there were no abnormal physical signs. The plasma albumin did not at first decrease, but was 1·8 g. per 100 ml. when the renal biopsy was performed on 14.5.57. The serum cholesterol was increased on admission, and was at the same level in May. He has subsequently returned to full work. When he was last seen 26.9.57 there were no abnormal physical signs. The blood-pressure was 120/80, blood urea 32 mg. per 100 ml., and plasma proteins below normal. The serum cholesterol had fallen to 327 mg. per 100 ml. Fairly heavy proteinuria was still present.

Conclusion. Nephrotic syndrome associated with focal glomerulonephritis; possible evidence of a sensitization phenomenon.

Patient 17, a man aged 55, was admitted to hospital on 11.4.57, with a history of having lost 35 lb. in weight during the previous three months. No specific symptoms occurred until five weeks before admission, when he developed a sore throat associated with conjunctivitis and malaise, followed shortly by stabbing pains in the left loin, aggravated by breathing. He continued his work, but was troubled by night sweats and progressive lethargy. During the 10 days before admission he developed rapidly increasing swelling of the legs and lower trunk, with breathlessness. Painless haematuria had been present during the previous week. There was no relevant previous history.

On examination he was a rather heavy man, with gross oedema of the legs and some oedema of the trunk. The blood-pressure was 210/120, pulse-rate 80, and venous pressure raised. The urine contained fairly large amounts of protein, and large numbers of red cells in the sediment. The antistreptolysin-O titre was 220 units per ml. No L.E. cells were seen on repeated examination. In the course of three weeks the patient's blood-pressure fell to normal, and he lost his oedema. Renal biopsy was performed on 28.5.57. He was not given any specific therapy, and was discharged on 1.6.57. When last seen on 15.8.57 he was very well. There was no oedema, the blood-pressure was 135/80, and the urine contained a trace of protein, with moderate numbers of red cells in the sediment.

Conclusion. A nephrotic syndrome with histological changes characteristic of a focal glomerulonephritis. The onset possibly followed a sore throat.

Patient 18, a man aged 51, in June 1956 developed a sore throat and a dis-

charge of thick yellow mucopus from the nose, which lasted for three weeks. In July 1956 swelling of the legs began, and rapidly progressed to a gross oedema. He was admitted to hospital two weeks after the first onset of swelling. There was no relevant past history, except a possible cystitis in 1942; the urine was said to have been quite normal after this episode.

On examination there was gross oedema involving the entire body. The blood-pressure was 160/85. Venous pressure was not raised. Renal biopsy was performed on 21.8.56. He was treated with a short course of prednisolone, 40 mg. four times a day, but developed severe psychotic symptoms, and the drug was stopped at once. A profuse diuresis followed immediately after stopping the prednisolone, the patient losing 20 lb. in four days. Subsequently the proteinuria gradually decreased, and the plasma proteins reverted to normal. A second renal biopsy was performed on 22.2.57. He was last seen in September 1957, over a year after the onset of the original illness, when he was completely well. There were no abnormal physical signs, and no proteinuria.

Conclusion. Nephrotic syndrome with complete remission. Histological changes mainly confined to the tubules. Aetiology unexplained.

Patient 19, a woman aged 81. In August 1955 she developed increasing oedema of the legs, eventually involving the trunk, and was admitted to hospital. At the age of eight this patient had had osteomyelitis of the right ankle, with a persistent discharge. At the age of 12 the right foot was amputated. Periodic discharge from the amputation stump led to a mid-thigh amputation of the right leg at the age of 21. There was no further discharge or trouble.

On examination there was gross oedema, the blood-pressure was 145/70, and there was very heavy proteinuria. A urinary infection was treated with sulphadimidine. Repeated mersalyl injections failed to produce any diuresis. Towards the end of November the patient was still grossly oedematous. Venous pressure was not raised. The urine contained a heavy growth of enterococci, and she was treated with a course of terramycin. At the same time a fairly rapid diuresis started, and the patient eventually lost all her oedema. A renal biopsy was performed on 6.1.56, during the diuresis. So far as is known from a rather inadequate follow-up, there has been no return of oedema, and the urine was free of protein in September 1957.

Conclusion. Nephrotic syndrome with complete remission. Minimal histological changes confined to the tubules. Aetiology unexplained.

Patient 20, a young married woman aged 19. During the second month of her first pregnancy she developed swelling of the face and legs, and was admitted to another hospital in December 1956; her last menstrual period was on 8.10.56. There was generalized oedema. The blood-pressure was 135/70. The urine contained 5 g. to 16 g. of protein in 24 hours. The blood urea was 10 mg. per 100 ml., serum cholesterol 190 mg. per 100 ml., and total plasma proteins 4.0 g. (albumin 2.0 g.) per 100 ml. The patient was kept in hospital, and at first treated with a low-sodium diet and katonium resin. Oedema was mainly related to the patient's activity, and was present when she was allowed to be up and about. From 20.2.57 prednisolone, 20 mg. thrice daily, was given. On 26.3.57 she was transferred to St. Mary's hospital. Renal biopsy was performed on 2.4.57, when there was slight oedema. In view of the findings from renal biopsy, prednisolone therapy was slowly withdrawn, and the patient allowed to continue her pregnancy normally at home, the positive therapy being an additional protein intake of 30 g. per day. The patient did not take this supplement regularly. The pregnancy continued normally, with diminishing proteinuria and no oedema.

On 18.7.57, four days before the expected date of delivery, she was admitted with ruptured membranes. Two hours after her admission foetal distress was diagnosed, and an emergency Caesarian section was performed. A live female child, weighing 6 lb. 1 oz., was delivered. Both mother and child did well. Full breast feeding was carried out. The second renal biopsy was performed on 24.9.57. The patient was very well, still breast-feeding her infant. There was no oedema, the blood-pressure was 104/72, and there was no proteinuria.

Conclusion. Unexplained transient nephrotic syndrome arising during pregnancy.

Methods

Renal biopsy was performed by the percutaneous route with a modified Vim–Silverman needle; the technique was that described by Kark and Muehrcke (1954). Biopsy material was immediately fixed in 10 per cent. formol saline, and in some cases also in cold acetone for enzyme studies. Sections were cut serially at 5 μ thickness, and mounted six per slide. Most slides were stained with haematoxylin and eosin, while at least one slide from each patient was stained with van Gieson's mixture, periodic-acid-Schiff reagent, and methyl violet for amyloid. Slides for enzyme studies were prepared in low-temperature wax, and stained for alkaline phosphatase by the coupling azo dye method (Pearse, 1953).

Pathological Findings

A summary of the main pathological changes in the kidney in all 20 patients is given in Tables II and III.

Cases 1 to 8 showed the histological changes of Ellis type 2 nephritis, the fundamental pattern being similar in all. The earliest glomerular change was seen in the first biopsy of Case 3, in which scattered glomeruli showed thickening of occasional capillaries, some glomeruli appearing normal. Tubular changes consisted of mild dilatation of proximal convoluted tubules, which were lined by finely granular cubical cells, scanty casts being present in the distal tubules. Cases 7 and 8 showed rather more advanced glomerular changes, thickening of the basement membrane being more widespread, and many more nuclei were present in the tufts of Case 7. The second biopsy from Case 3, and the only biopsy from Case 4 showed the next change in the process of glomerular involvement. All the glomeruli were affected by a diffuse eosinophilic thickening of the basement membrane (Plate 49, Fig. 1), all the capillaries being affected. The capillary lumina were held patent and had a very uniform internal diameter, but in spite of this contained very few red blood-cells. Proximal tubules were lined by columnar, finely granular, eosinophilic cells, and interlobular arteries showed a mild intimal thickening. The first biopsy from Case 2, and the only biopsy from Case 5, showed a more advanced glomerular lesion. Thickening of the basement membrane was more pronounced (Plate 49, Figs. 2 and 3), the capillary lumina being patchily reduced in Case 5. This reduction was due also to cytoplasmic swelling of the endothelial cells. There was an early solidification of the tufts of

TABLE II

Pathological Changes in the Glomeruli

Patient number	*Number of glomeruli seen*	*Cellularity of tuft*	*Patency of capillaries*	*Thickening of basement membrane and distribution**	*Solidification of tuft**	*Adhesions between tuft and Bowman's capsule*	*Other changes*
1	50	Increased	Reduced	Extreme G.D.	Moderate G.D.	Occasional	..
2a	30	Normal	Patent	Moderate G.D.	Early G.D.	Moderate	Pseudo-tubules in Bowman's space
2b	12	Decreased	Reduced	Extreme G.D.	Extreme G.D.	Frequent	..
3a	9	Normal	Patent	Mild F.L.	Absent	Occasional	..
3b	20	Normal	Patent	Moderate G.D.	Early F.L.	Occasional	Occasional reduction of capillary lumen
4	48	Normal	Patent	Moderate G.D.	Early F.L.	Absent	Tuft lobulation accentuated
5	24	Normal	Reduced	Moderate G.D.	Early G.D.	Occasional	Some capillaries patent
6	3	Reduced	Reduced	Extreme G.D.	Extreme G.D.	Absent	Very few glomeruli seen
7	8	Increased	Patent	Mild G.D.	Absent	Occasional	Some cytoplasmic swelling of cells
8	30	Normal	Patent	Mild G.D.	Absent	Absent	..
9	28	Normal	Patent	Absent	Ischaemic F.D.	Occasional	Occasional ischaemic glomeruli
10	32	Normal	Patent	Absent	Ischaemic F.D.	Occasional	Some glomeruli ischaemic
11	26	Normal	Patent	Mild F.L.	Ischaemic F.D.	Occasional	Occasional ischaemic glomeruli
12	26	Normal	Patent	Mild G.D.	Absent	Absent	Capillaries widely patent with rigid walls
13	14	Increased	Patent	Mild G.D.	Absent	Absent	Capillary walls rigid
14	18	Normal	Patent	Moderate F.L.	Moderate F.L.	Frequent	'Wire - loop' - like lesion in one tuft. Peripheral hyalinization of some glomeruli
15	18	Mild increase	Patent	Mild F.L.	Moderate F.L.	Moderate	Peripheral hyalinization of lobules in occasional glomeruli
16	27	Increased	Patent	Absent	Mild F.L.	Moderate	Peripheral hyalinization of scattered glomeruli
17	28	Increased	Patent	Mild F.L.	Mild F.L.	Moderate	Increased lobulation of tufts
18a	15	Normal	Patent	Absent	Absent	Absent	..
18b	18	Normal	Patent	Mild F.L.	Absent	Occasional	Very minor local thickening of basement membrane in few tufts
19	9	Normal	Patent	Absent	Absent	Absent	Occasional ischaemic golmerular changes
20a	24	Normal	Patent	Absent	Absent	Absent	Capillaries widely patent with rigid basement membranes. Swelling of endothelial and epithelial cells
20b	18	Normal	Patent	Absent	Absent	Absent	Prominent rigidity of basement membranes

* F = Focal; refers to changes affecting only scattered glomeruli.
G = Generalized; refers to changes affecting all glomeruli.
L = Local; refers to changes affecting only a portion of the tuft.
D = Diffuse; refers to changes affecting the whole tuft.

scattered glomeruli, caused partly by the basement-membrane thickening and partly by the appearance of periodic-acid-Schiff staining fibres in what corresponded to the intercapillary space. Some adhesions were present between the

TABLE III

Changes in Tubules, Interstitium, and Blood-vessels

Patient number	*Proximal convoluted tubules (A.P. = alkaline phosphatase)*	*Remainder of nephron*	*Interstitium*	*Vessels*
1	Most lined by columnar eosinophilic granular epithelium. Few atrophic	Scanty casts	Fine fibrosis Foam cells	Normal
2a	As for Case 1	Scanty casts	Oedema. Lymphocytes. Eosinophils	Normal
2b	As for Case 1, but more atrophic, and some lost. Good A.P. pattern in normal tubules	Scanty casts	Fine fibrosis	Mild arteriolar hyalinization
3a	As for Case 1, with dilatation	Scanty granular casts	Normal	Normal
3b	As for Case 1	Scanty casts	Normal	Mild intimal arterial thickening
4	As for Case 1, with some dilated	Scanty casts	Mild oedema	Mild intimal arterial thickening
5	One-third normal. Others early atrophy. Poor A.P. pattern	Scanty casts	Fine fibrosis. Giant-cell reaction. Haematoxyphile 'lakes'	Mild arteriolar hyalinization
6	Considerable atrophy and loss	Many casts	Fibrosis Lymphocytes	Hyaline arterioles. Intimal arterial thickening
7	As for Case 1. A.P. pattern good	Scanty casts	Oedema	Normal
8	Lined by low granular epithelium	Scanty casts	Normal	Normal
9	Many atrophic or lost	Moderate casts	Fibrosis. Lymphocytes	Hyaline arterioles. Intimal arterial thickening
10	Many normal, with good A.P. pattern. Some atrophic. Some lost	Scanty casts	Fibrosis. Lymphocytes	As for Case 9
11	Many normal. Some atrophic. Some lost	Many casts	Fibrosis. Lymphocytes	As for Case 9
12	Profound atrophy and loss. Few normal	Scanty casts	Fibrosis. Lymphocytes	Normal
13	Moderate atrophy and loss. Poor A.P. pattern	Moderate casts and nuclear debris	Fine fibrosis	Hyaline arterioles and intimal thickening of arteries
14	Dilated and lined by cubical cells	Moderate casts	Scattered lymphocytes	Normal
15	Many normal. Some atrophic	Occasional red cells	Mucinous change	Normal
16	Many normal. Good A.P. pattern	Normal	Normal	Normal
17	Dilated, with low epithelium. Poor A.P. pattern	Hyaline and pigment casts	Oedema. Lymphocytes	Mild arteriolar hyalinization
18a	Most normal. Some lined by low epithelium	Few casts	Normal	Mild intimal arterial thickening
18b	Most normal. Good A.P. pattern	Few casts	Normal	As for Case 18a
19	Most normal. Few atrophic. Fine brown granules in some cells	Few casts	Normal	As for Case 18
20a	Normal. Good A.P. pattern	Few casts	Normal	As for Case 18
20b	Normal. Good A.P. pattern	Few casts	Normal	As for Case 18

tuft and Bowman's capsule, and in the first biopsy of Case 2 epithelial hyperplasia gave rise to a pseudo-tubular pattern. Tubular changes in Case 2 were not pronounced, except that some proximal tubules were lined by flattened epithelium. The interstitial tissues showed oedema and infiltration by lymphocytes and eosinophils. In Case 5 more tubules were atrophic, but casts were scanty. The interstitial tissue showed 'lakes' of haematoxyphile material, in which were finely particulate structures. There was also a foreign-body giant-cell reaction to

elongated clefts, possibly of cholesterol origin, although it was not possible to confirm this explanation because of lack of material. Hyalinization of arterioles and intimal arterial thickening were also found. Case 1 showed an even more advanced lesion in the glomeruli, which appeared solid, the nuclei often being collected at the centres of lobules (Plate 49, Fig. 4). Capillary lumina were considerably reduced. Most proximal tubules were lined by finely granular columnar cells, only a few being atrophic, while 'foam cells' were present in the interstitium (Plate 50, Fig. 5). The second biopsy from Case 2 and the only biopsy from Case 6 showed all glomeruli to have a solidified hyaline appearance (Plate 50, Fig. 6) which, certainly in the former, could be seen to be caused by an increase in fibres in the intercapillary space, as well as by thickening of the basement membrane. The glomeruli were bloodless. Many proximal tubules had disappeared, and many were atrophic. Casts were present in the distal nephron, while there was a fine interstitial fibrosis and arteriolar hyalinization.

Three cases (9, 10, and 11) showed changes which had many features of benign nephrosclerosis. Most glomeruli were normal, although in Case 10 occasional capillaries and scattered tufts showed early thickening of the basement membrane. Scattered glomeruli in all three cases showed ischaemic changes, namely the presence of a hyalinized tuft surrounded by a mantle of collagen. In Case 9 tubular changes were pronounced, many proximal tubules being lined by low epithelium (Plate 50, Fig. 7), and some tubules had disappeared. Similar, though less severe changes, were present in Cases 10 and 11. Many normal tubules were present in all three. All showed prominent hyalinization of afferent arterioles and intimal thickening of interlobular arteries. Fibrosis and scattered lymphocytes were present in areas of tubular loss.

Two cases (12 and 13), diagnosed clinically as renal vein thrombosis, showed great similarities. The main change was an early but undoubted thickening of the basement membrane, occurring diffusely within the tuft, all glomeruli being affected (Plate 50, Fig. 8). The capillaries were widely patent, and had a rigid appearance. Bowman's space was dilated in Case 12. Tubular changes were conspicuous, especially in Case 12, in which most proximal tubules were atrophic (Plate 51, Fig. 9) and lined by flattened epithelium which stained basophilically, casts being present in the distal tubules. A similar, although not so pronounced, tubular atrophy was found in Case 13. The interstitium showed a fine fibrosis in both cases, and in Case 13 there was hyalinization of arterioles and intimal thickening of arteries.

Case 14, which clinically had many of the features of systemic lupus erythematosus, showed changes in half the glomeruli. These consisted of hyalinization of the periphery of individual lobules of glomerular tufts, often causing adhesion to Bowman's capsule, nuclei being increased in these areas. There was a patchy moderate thickening of the basement membrane in half the tufts, and one glomerulus showed a pronounced localized and eosinophilic thickening of the basement membrane, resembling the 'wire-loop' lesion of systemic lupus erythematosus (Plate 51, Fig. 10). No haematoxyphile bodies were seen. Tubules were dilated, but not otherwise remarkable, a moderate number of casts being found

in the distal nephron. Cases 15 and 16 showed some of the features of the previous case, except that no lesions resembling 'wire loops' were seen. Both showed changes in over half the glomeruli seen, others being normal. The change consisted of a hyalinization of the periphery of the glomerular tuft, with frequent adhesion to Bowman's capsule (Plate 51, Fig. 11). Sometimes only a single lobule was affected. A local type of basement-membrane thickening was found in affected tufts in Case 15. In both cases certain tufts showed a localized increase in cellularity, and both showed an isolated glomerulus with what resembled an organized crescent. In Case 15 there were some atrophic proximal tubules, the great majority in both cases being eosinophilic and finely granular. Case 17 showed similar features in many respects, except that there were more nuclei and less solidification in the tufts, the picture again being that of a focal glomerulonephritis (Plate 51, Fig. 12). One glomerulus showed a structure resembling a fibrotic crescent.

Two biopsies from Case 18 and one from Case 19 showed very few abnormalities. Case 18 showed an equivocal thickening of the basement membrane in scattered glomeruli in the second biopsy, while in Case 19 the glomeruli were normal, except one which was ischaemic. The proximal tubules showed a lower type of epithelium in a minority of tubules, the epithelial cells otherwise being tall and granular. In Case 19 there were collections of fine brown pigment in tubular cells. The biopsy from Case 20 showed a striking patency of the glomerular capillaries, the basement membranes appearing rigid but not convincingly thickened. There was cytoplasmic swelling of both epithelial and endothelial cells in the glomeruli, the tubules showing no remarkable changes. A second biopsy six months later showed the basement-membrane changes unaltered, but the cytoplasmic swelling had disappeared.

Unfortunately there was insufficient material for frozen sections to be examined for lipid. Enzyme staining for alkaline phosphatase gave results shown in Table III.

Clinical Assessment

The clinical assessment of this group of 20 patients with a nephrotic syndrome is inevitably unsatisfactory in view of the short follow-up period. It is further complicated by differences in therapy. Nevertheless, a review of these patients does not seem to be premature, as the histological evidence, set beside the clinical course, suggests that a considerable proportion of patients with a nephrotic syndrome may have pathological lesions other than those commonly accepted as being associated with this clinical presentation. More important, possibly, the histological appearances may, with further experience, prove to be of prognostic value. It was not possible with any certainty to predict the histological appearances in any case, nor did this become easier with more experience.

The patients can be placed in two main groups, those with the histological picture of type 2 nephritis (Cases 1 to 8) and those not showing such changes. Of the eight patients in whom the type 2 changes were found, none has shown a

complete remission with disappearance of proteinuria. Four have had considerable nitrogen retention over prolonged periods (Cases 1, 2, 3, and 6), two patients (Cases 2 and 3) showing a rapidly progressive lesion observed from near the beginning of their illness. Of the 12 patients with histological changes other than those of type 2 nephritis, four have shown complete remission, losing their proteinuria as well as oedema, the biochemical changes in the plasma reverting towards normal (Cases 9, 18, 19, and 20). Of the remaining eight patients in this group, two were thought to have renal vein thrombosis. In Case 12 there was clinical evidence of collateral venous flow on the trunk to support the diagnosis of inferior venacaval thrombosis, and in Case 13 there was clinical evidence of thrombotic episodes in the cerebral and pulmonary blood-vessels, such as have been described in proven cases of renal vein thrombosis. Four patients (Cases 14, 15, 16, and 17) showed a histological picture of focal glomerulonephritis; this histological finding in renal biopsy material is more fully discussed elsewhere (Heptinstall and Joekes, 1958). One patient (Case 14) presented some clinical features of systemic lupus erythematosus, but no L.E. cells were found on repeated examination. No follow-up was possible in this patient. Of the other three, in one (Case 15) the onset of a nephrotic syndrome was associated with a Schönlein–Henoch syndrome; when the patient was seen five months after the onset of his illness the only definite abnormality was the persistence of proteinuria. From our own experience with renal involvement in this syndrome, and from some previous reports (Bywaters, Isdale, and Kempton, 1957) it is likely that the proteinuria will gradually disappear during the next year or so. In Case 17 only a trace of proteinuria remained five months after the patient's admission to hospital. In Case 16 proteinuria still persisted seven months after the onset, but there was no oedema, and the biochemical changes had almost returned to normal. In Case 10 the nephrotic syndrome was thought to be due to a toxic lesion following long-term treatment with mercury; in this patient the follow-up period was short, but Case 9, with a very similar presentation, had eventually shown a complete remission. Both these patients showed vascular changes characteristic of benign nephrosclerosis, which were also present in Case 11. In Case 11 proteinuria was steadily diminishing up to 16 months after the first known finding of proteinuria, but there had been little evidence of biochemical abnormalities.

Pathological Discussion

Eight of the 20 cases showed the picture of type 2 nephritis (Ellis, 1942), and in two cases a second biopsy showed an extension of the glomerular change. The most constant feature was thickening of the basement membrane in the capillary tufts of the glomeruli, a feature well described by Bell (1929) and Dunn (1934). In the well-developed stage the thickening affects all the glomeruli in a diffuse manner, but in the first biopsy of Case 3 only a proportion of the glomeruli, and in these not all the basement membranes, are affected. This feature has recently been stressed by Rich (1957), who also demonstrated in autopsy material the

more severe involvement of the juxtamedullary glomeruli. The finding that the earliest lesion was not generalized may make it difficult to forecast which case is an example of type 2 nephritis, although the diagnosis is unmistakable in well-developed cases. The rate of progression can be very rapid, and a second biopsy four months later in Case 3, and a second biopsy eight months later in Case 2, showed a much more advanced picture. A second biopsy in six months may well give information which is lacking on the first occasion. Tubular changes are not prominent, and in only two biopsies, the second in Case 2, and in Case 6, was there any appreciable tubular atrophy. These cases were those in which glomerular hyalinization was profound. The usual picture is that of rather plump epithelial cells in the proximal tubules having a finely granular appearance. Alkaline phosphatase activity was good in two of the cases in which it was tested, and poor in one. In the latter case it must be added that the investigation was done before we started using low-melting-point wax in the processing. Eosinophilic casts were scanty in the distal part of the nephron in all cases, except that which showed severe glomerular changes and tubular atrophy. We were unable to stain for lipid because of the small amount of material available, but Case 1 showed foam cells in the interstitium, and Case 5 showed a foreign-body giant-cell reaction to elongated clefts, possibly of cholesterol origin.

Three cases (9, 10, and 11) showed an appearance similar to that of benign nephrosclerosis, with scattered hyalinized glomeruli surrounded by collagen. The unaffected glomeruli showed no unequivocal thickening of the basement membrane, except in one case which showed localized thickenings in scattered glomeruli. The changes do not appear to be those of a type 2 nephritis at an early stage, and the subsequent clinical progress supports this view. There was tubular damage of a severe degree in one of the patients, and of a lesser degree in another, both having received mercurial treatment. The possibility that mercury caused tubular damage must be seriously considered.

Two cases were clinical examples of renal vein thrombosis. Both showed a diffuse thickening of the basement membrane in all glomeruli, with pronounced tubular atrophy. Thickening of the basement membrane is present in cases of renal vein thrombosis (Blainey, Hardwicke, and Whitfield, 1954; Pollak, Kark, Pirani, Shafter, and Muehrcke, 1956) and the question arises whether such cases represent renal vein thrombosis occurring in a type 2 nephritis, a sequel known to occur with amyloidosis of the kidney, or whether the glomerular changes are secondary to the thrombosis. In the two cases under discussion a remarkable feature is the dissociation between tubular changes, which are moderate or severe, and the glomerular changes, in which there is no reduction of the lumina, a combination unlike that of a classical type 2 nephritis. For this reason we would not classify these cases as examples of this type of nephritis.

Case 14 is clinically an example of systematic lupus erythematosus, although L.E. cells have never been seen, and it is impossible to persuade the patient, now in remission, to attend hospital. One glomerulus shows a lesion resembling a 'wire loop', and others show hyalinization in individual lobules of the tuft, suggestive of a healed local glomerulitis such as is seen in systemic lupus erythe-

matosus (Klemperer, Pollack, and Baehr, 1941; Muehrcke, Kark, Pirani, and Pollak, 1957; Everall, Heptinstall, and Joekes, 1958).

Cases, 15, 16, and 17 are classified together because of certain similarities in glomerular changes. There is endothelial and epithelial cellular proliferation in the tufts, collections of peripheral hyaline in individual lobules, and what appear to be organized crescents. All three show moderate numbers of adhesions between the tuft and Bowman's capsule. We interpret these cases as being examples of a focal type of glomerulonephritis.

The remaining three cases show remarkably few changes, there being, in particular, no thickening of the basement membrane in the glomeruli, except for the second biopsy in Case 18, in which there was a very mild focal and local thickening. A conspicuous feature of Case 20 was the patency and rigidity of the glomerular capillaries without unequivocal thickening of the wall. No tubular abnormalities are seen in any of these cases, alkaline phosphatase activity being good in the three biopsies in which it was tested.

General Discussion

Of the 20 patients, only eight show a histological picture of type 2 glomerulonephritis, and in two the findings are consistent with renal vein thrombosis. In none of these patients is there evidence of remission. In another patient the histological appearances are consistent with systemic lupus erythematosus; unfortunately no follow-up details are available. Of the other nine patients, four have had complete remissions, and in one a trace of protein is present in the urine as the only remaining abnormality; in none of the other four is there evidence of progression of the renal lesion, and where any significant change has occurred it is to show improvement, both clinically and bicchemically.

In a recent review of the results of renal biopsy in 13 cases of the nephrotic syndrome (Ross and Ross, 1957) two showed the presence of amyloid disease, six cases showed what was accepted as the characteristic picture of a type 2 nephritis, and in the remaining five the general description was given as follows: '. . . histological lesions were scanty; in the glomeruli no thickening of the basement membrane and no intercapillary hyalinization were found, but slight increase in the cellularity of the glomerular tuft, capsular adhesions, and "bland" focal lesions of the tuft were seen'. These changes were regarded by the authors as evidence of early type 2 nephritis because 'in their clinical course, so far as it has been followed, they closely resembled other patients in whom the diagnosis of type 2 was proved subsequently at necropsy'. In this latter group the changes are very similar to those found in our patients with what we have termed focal glomerulonephritis. Unfortunately Ross and Ross (1957) presented very brief clinical details of only four of the nephrotic patients. Of these four, two showed typical changes of type 2 nephritis, the other two showed focal lesions; one of the latter died of intercurrent infection, but the other showed a complete remission after steroid therapy. It might be inferred from the histological interpretation by the authors that an early type 2 nephritis can be cured by steroid

therapy, or may resolve spontaneously. A more likely interpretation in our view is that a nephrotic syndrome may occur with a variety of renal lesions, not necessarily showing any glomerular involvement; some of these are reversible—probably lesions other than those of type 2 nephritis.

Pollak, Kark, Pirani, Soothill, and Muehrcke (1957) referred to 56 biopsies in patients with a nephrotic syndrome. The great majority of their patients are stated to have shown lesions of type 1 or type 2 glomerulonephritis, and a few of these showed a mixed histological picture, with characteristics of both types. Other lesions found were diagnostic of, or consistent with, amyloidosis, renal vein thrombosis, systemic lupus erythematosus, and diffuse diabetic glomerulosclerosis. One group of six patients showed no organic glomerular changes, but the glomeruli contained many red cells; the tubules showed severe degenerative changes, with a low epithelium. These patients all responded with a good diuresis to corticotrophin therapy, and were subsequently 'clinically normal'. The clinical course and response to corticotrophin therapy in these patients were not compared with those found in patients with glomerular lesions. Bjørneboe, Brun, Gormsen, Iversen, and Raaschou (1952) reported biopsy studies in eight patients with a nephrotic syndrome; in four of these the changes were characteristic of amyloidosis, in two of chronic glomerulonephritis, and two showed no glomerular changes. In discussing the response to corticotrophin therapy the authors pointed out that the most favourable clinical response was obtained in the two patients who showed no glomerular changes. The ultimate prognosis was not discussed. The degree of proteinuria when the patients were first seen appeared to the authors to be inversely related to the severity of glomerular involvement. It has been argued by inference that the similarity of the differential pattern of plasma-protein concentrations in the oedema fluid and urine of nephrotic patients is consistent with impairment of tubular reabsorption, which would thus account for the proteinuria in the nephrotic syndrome (Freeman and Joekes, 1957). If this view is acceptable, there is naturally nothing surprising in the absence or paucity of glomerular lesions in some instances of the nephrotic syndrome. The presence of so-called proteinaceous material in Bowman's capsule in our cases was a rarity. Ross and Ross (1957) stated that they 'attach little diagnostic importance to tubular changes . . .' in cases without the typical type 2 lesions. In our series there were, however, patients in whom the predominant change was in the tubules, and in some of these a complete remission has occurred.

There seems to be no real justification for trying to fit the histological appearances in renal biopsy material obtained early in the course of renal disease into classifications drawn from post-mortem material. With the short follow-up period in our patients it is realized that the possibility of further relapses cannot be excluded. Nevertheless, the four patients who have had a complete remission lasting to the time of writing all showed the absence of the histological changes characteristic of type 2 nephritis. We know of no direct evidence that steroid therapy has cured patients proved to have type 2 nephritis. In reviews of patients with a nephrotic syndrome treated by steroids, very variable figures for

complete remissions are given, but no correlation of renal histology with the clinical course has been possible before the use of needle biopsy. There does not seem any doubt that steroid therapy can induce a water and salt diuresis in patients with a nephrotic syndrome, and that maintenance therapy may control accumulation of oedema. The main aim of therapy, however, must be to find a cure, preventing progressive renal damage. So long as it is claimed that steroid therapy affects such a cure in a proportion of cases, the active search for other possible therapeutic approaches will seem less pressing.

We are grateful to the following physicians for referring patients to us, or allowing us access to them: Doctors W. D. W. Brooks, N. F. Coghill, R. D. Green, M. Hamilton, Q. J. Hobson, F. Avery Jones, Idris Jones, K. D. Keele, J. W. Litchfield, R. J. Porter, and C. A. Young; Professor W. S. Peart, Professor Sir George Pickering, and Professor Hulst of Utrecht. We are especially indebted to Mr. A. Beasley for the preparation of large numbers of serial sections essential in the present investigation. One of the authors (A. M. J.) was in receipt of a research grant from St. Mary's Hospital.

Summary

1. Twenty-four renal biopsies have been performed by the percutaneous route in 20 adult patients with a nephrotic syndrome.

2. The histological material has been analysed, and on this basis the cases have been separated into several groups: type 2 nephritis, renal vein thrombosis, focal glomerulonephritis, benign nephrosclerosis, probably chronic toxic tubular damage and, lastly, cases with no apparent abnormalities. It was not possible to predict the histological appearance on clinical grounds.

3. Of the eight patients with type 2 nephritis and the two with renal vein thrombosis, none has shown a complete remission. One of the patients in the group showing focal glomerulonephritis was thought on clinical grounds to have systemic lupus erythematosus. Of the remaining nine patients four have shown a complete remission, and a fifth has a trace of proteinuria as the only remaining abnormality.

4. It is suggested that a histological picture other than that usually recognized as associated with a nephrotic syndrome is quite common, and that atypical histological appearances should not be labelled as type 2 nephritis.

5. Renal biopsy seems essential for assessment of therapeutic measures in type 2 nephritis.

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FIG. 1. Glomerulus showing mild to moderate thickening of the capillary basement membrane. Second biopsy from Case 3 (haematoxylin and eosin, ×240)

FIG. 2. Glomerulus with moderate diffuse thickening of the capillary basement membrane and surrounding cellular reaction. First biopsy from Case 2 (haematoxylin and eosin, ×300)

FIG. 3. Glomerulus with moderate diffuse thickening of the capillary basement membrane. The only biopsy from Case 5 (haematoxylin and eosin, ×240)

FIG. 4. Glomerulus with prominent solid lobules, one showing an increased number of cells. The only biopsy from Case 1 (haematoxylin and eosin, ×210)

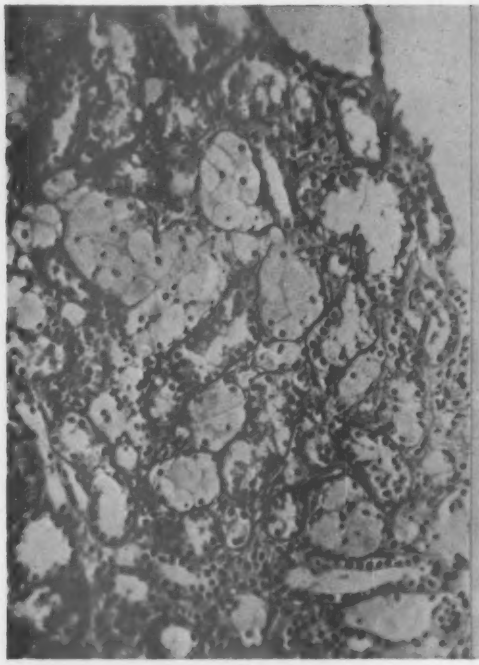

FIG. 5. Collections of 'foam cells' in the interstitium, from Case 1 (haematoxylin and van Gieson's stain, ×170)

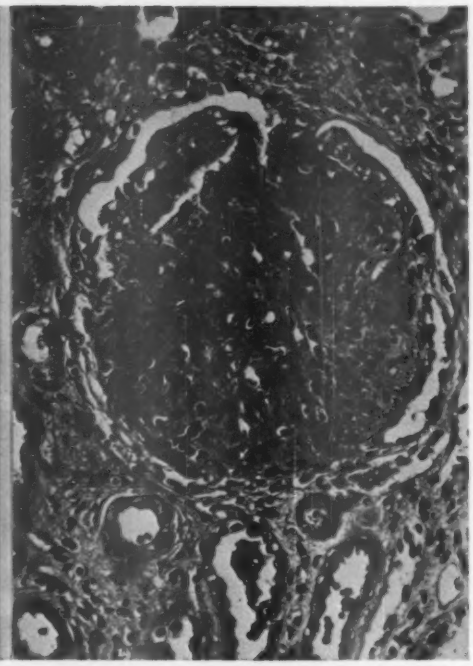

FIG. 6. Solidified hyaline glomerulus from the second biopsy in Case 2 (haematoxylin and eosin, ×240)

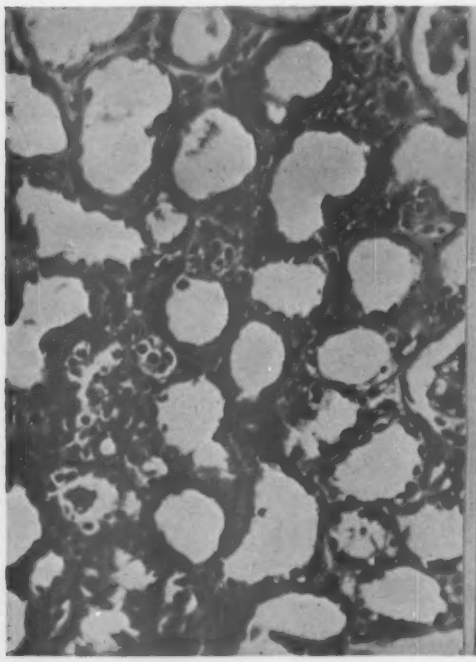

FIG. 7. Proximal convoluted tubules lined by flattened epithelium, from Case 9 (haematoxylin and eosin, ×240)

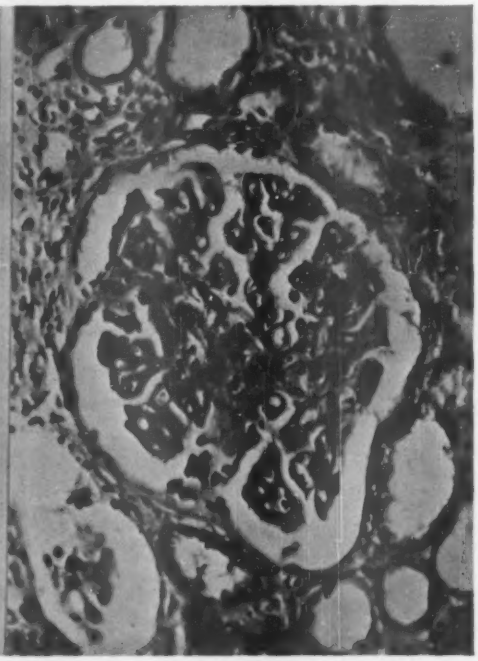

FIG. 8. Glomerulus showing early diffuse thickening of the capillary basement membrane. The only biopsy from Case 12 (haematoxylin and eosin, ×240)

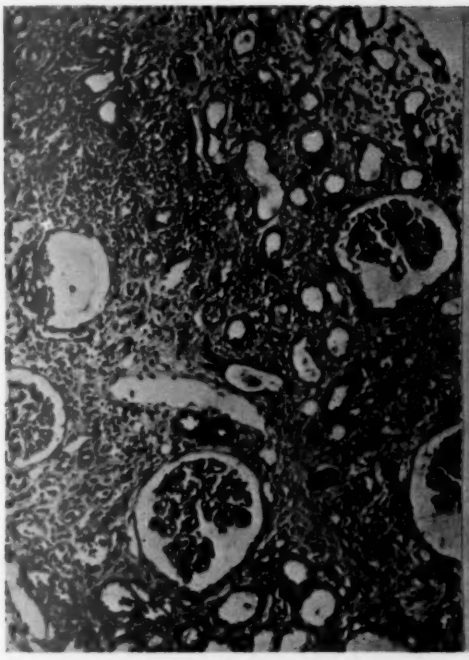

FIG. 9. Atrophy and loss of proximal convoluted tubules, with lymphocytes in the interstitial tissue, from Case 12 (haematoxylin and eosin, ×100)

FIG. 10. Glomerulus with lesion resembling 'wire loops', from Case 14 (haematoxylin and eosin, ×400)

FIG. 11. Glomerulus with peripheral area of hyalinization and adhesion to Bowman's capsule, from Case 15 (haematoxylin and eosin, ×300)

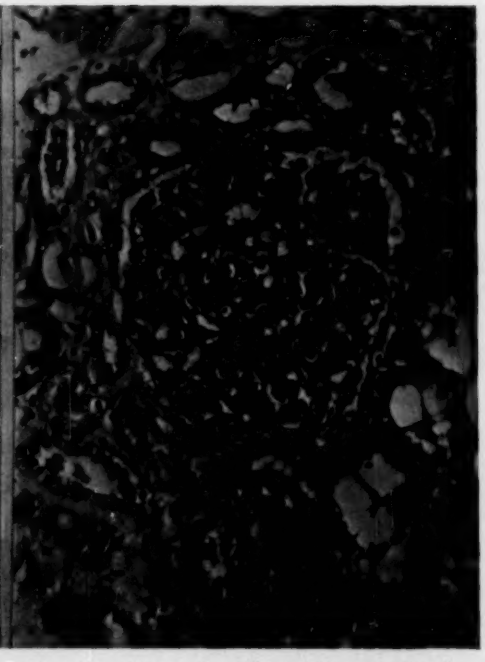

FIG. 12. Glomerulus showing increased cellularity, from Case 17 (haematoxylin and eosin, ×240)

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SUBACUTE COMBINED DEGENERATION OF THE SPINAL CORD IN NON-ADDISONIAN MEGALOBLASTIC ANAEMIA[1]

By JOHN RICHMOND and STANLEY DAVIDSON

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With Plates 52 to 55

The term 'subacute combined degeneration' (SCD) is used to describe a disease of the spinal cord, invariably associated with peripheral neuritis, in which the white matter of the posterior and lateral columns is predominantly affected. This cumbersome title was gradually evolved at the end of last century: 'subacute' because, as Russell (1898) pointed out, one of the features which distinguished the 'new' disease from the chronic ataxic paraplegia of Gowers (1886) was its more rapid course and fatal termination; 'combined' because tracts of different function were usually damaged, and 'degeneration' because that was believed to be the nature of the histological changes in the spinal cord.

The earliest accounts of SCD were probably those of Leichtenstern (1884) and Lichtheim (1887). Both authors emphasized the unique association of 'pernicious anaemia' with spinal cord degeneration. Leichtenstern, however, stated that neither of his cases showed atrophy of the gastric glands, a finding which would exclude the diagnosis of Addisonian pernicious anaemia by modern criteria. Russell, Batten, and Collier (1900) attempted to characterize SCD by reviewing previous reports and by describing seven cases which they had studied personally during life and at autopsy. This report is still quoted as an authoritative account of the disease (Wilson, 1955; Brain, 1955), and a study of the clinical and pathological data given by the authors strongly supports their claim that their patients were suffering from subacute combined degeneration of the spinal cord. It is therefore surprising that, in all of the six patients in whom the blood was examined, the features were those of secondary anaemia, and not of pernicious anaemia. Furthermore, 'four of them had a definite history of syphilis, and in two only could this be excluded with comparative certainty'. Russell and his associates believed that SCD should be freed from its entanglement with anaemia, and that two totally distinct conditions had been confused. Confusion was to persist for many years, as is clearly indicated in a review of the subject in 1937 by Woltman and Heck, who found that no fewer than 60 causes had been advanced for 'combined degeneration just like that of pernicious anaemia'.

There may be two explanations for the divergence of opinion in earlier

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writings regarding the nature of SCD. First, modern haematological procedures were not available to the early investigators. There can now be little doubt that the great majority of cases of SCD are associated with Addisonian pernicious anaemia, a fact which makes it difficult to accept some of the observations in the early accounts of the disease. With the use of radioactive and microbiological techniques it has been established that the fundamental lesion in Addisonian pernicious anaemia is malabsorption of vitamin B_{12}, due to lack of intrinsic factor consequent on gastric atrophy. It is almost certain that vitamin-B_{12} deficiency is the main factor in the causation of SCD, since administration of vitamin B_{12} invariably halts progression of the spinal cord disease, and indeed may reverse some of the neurological signs, particularly if they are of short duration and due mainly to involvement of the peripheral nerves and posterior columns. It is well known that neurological changes may appear when anaemia is only mild or moderate in degree, or very rarely in its absence, a fact noted by Russell (1898), who pointed out that six previous authors had made the same observation. In such patients the level of vitamin B_{12} in the serum has been shown to be low, and malabsorption of radioactive-cobalt-labelled vitamin B_{12} has been demonstrated. Moreover, careful examination of the peripheral blood in these cases, even when the haemoglobin level is approximately within normal limits, will usually reveal a high colour index or raised mean corpuscular volume, indicative of macrocytosis, which can be corrected by vitamin-B_{12} therapy.

Secondly, peripheral neuritis is usually the first effect of vitamin-B_{12} deficiency on the nervous system. Peripheral neuritis is therefore almost always present when cord damage develops, and in the early stages of SCD this may give rise to considerable diagnostic difficulty. Failure to distinguish between the symptoms and signs of peripheral neuritis and those of damage to the posterior columns of the spinal cord may well account for some of the puzzling features in cases which have been reported in the past. Since the diagnosis of SCD depends on the demonstration of symptoms and signs due to involvement of the posterior or lateral columns of the cord, or the characteristic histological changes in the cord obtained at autopsy, or both, we consider it important to summarize the main clinical and pathological features of the disease.

Clinical Features of Subacute Combined Degeneration

Although the development of SCD is sometimes acute or subacute, the advent of symptoms is in the great majority of cases gradual and insidious. The clinical features of the fully developed syndrome are highly characteristic, but in the early stages of the condition, or when the lesions are confined to the posterior columns, the diagnosis may present the greatest difficulty. The lower limbs tend to be affected more than the upper limbs. It is common for the patient to be seen at first with paraesthesiae in the feet and legs in a symmetrical 'stocking' distribution, and with muscle weakness and unsteadiness in walking. There is also likely to be hyperalgesia of the muscles of the feet and calves, and

sensory loss of varying degree. Loss of vibration sense is extensive, and as the disease progresses all other forms of sensation become defective. Loss of appreciation of pain usually precedes loss of touch. In addition, the deep reflexes are usually depressed or absent. At this stage of the illness the symptoms and signs are indistinguishable from those of peripheral neuritis. In fact pathological studies show that lesions in the peripheral nerves and spinal cord usually coexist. To add to the diagnostic difficulty, it is noteworthy that, in the neuritis associated with SCD, the motor components of the peripheral nerves are very rarely affected. Profound loss of postural sensibility in the lower limbs, or a markedly disproportionate increase in the degree of impairment of muscle and joint sensibility and vibration sense, as compared with the degree of loss of superficial sensations, is strongly in favour of involvement of the posterior columns of the cord. The subsequent picture which is presented in an untreated case of SCD depends on whether the lesion affects the posterior or lateral columns or both, and on the relative amount of damage in the different tracts. It is usual for the features of posterior column involvement to appear first and to be the more severe. Both Cabot (1908) and Davidson (1957) found that in only a small proportion of their cases of SCD did signs of lateral column involvement predominate. Spastic paraplegia due to vitamin-B_{12} deficiency, without evidence of affection of the peripheral nerves or posterior columns, if it every occurs, must be very rare.

The classical picture of advanced SCD, which is seldom seen nowadays, is as follows: there is progressive paraplegia associated with Rombergism, ataxic gait, sensory loss, absent knee- and ankle-jerks, absent abdominal reflexes, and extensor plantar reflexes. Bladder disturbances occur late, precipitate micturition being succeeded by incontinence. Ocular symptoms associated with diplopia, nystagmus, and optic atrophy, are stated to be rare complications of SCD. Mental symptoms, varying from confusion to paranoid trends, are encountered more frequently. According to Holmes (1956), who recently reviewed the subject, unrecognized and untreated cerebral lesions may progress to severe dementia.

Pathological Features of Subacute Combined Degeneration

At the present time, even in units specializing in neuropathology, opportunity rarely arises *post mortem* to study the morbid changes in the nervous system due to SCD. Hence reliance must be placed on past records for descriptions of the pathological features of the condition. The essential lesions of SCD are found in the white matter of the thoracic segments of the spinal cord, the grey matter being but little affected. The histological changes consist of patchy degeneration of long fibre tracts. The degeneration first appears in the lower thoracic segments, as foci in the posterior and later in the lateral columns. The pathological process spreads locally and upwards and downwards in the cord from the thoracic level, by enlargement and coalescence of adjacent foci. The lesions tend to be situated near the periphery of the cord, and are irregular in shape on

transverse section. The first change is ballooning of the myelin sheaths, with lesser swelling of the axons. The greatly swollen myelin sheaths give a 'honeycomb' appearance to affected regions. Disintegration of the myelin sheaths and their contained axons is relatively slight, hence the amount of fatty breakdown products is less than might be expected from a superficial glance at sections of the cord. There is no inflammatory reaction and, in untreated cases, gliosis is minimal. It should be emphasized that when these changes are present the histological diagnosis of SCD presents little difficulty, but these are the findings in untreated cases of advanced disease. A similar but less extensive process of demyelinization may involve the peripheral nerves. In the brain two types of change have been described—chromatolysis of large nerve-cells, and demyelinization of the white matter of the cerebral hemispheres similar to the appearance seen in the cord.

Subacute Combined Degeneration in Non-Addisonian Megaloblastic Anaemia

Since the discovery of folic acid and vitamin B_{12} it has been possible to classify the megaloblastic anaemias, and in most instances to understand the mechanism of their production. It is not widely known that well-documented cases of SCD have been described not only in association with pernicious anaemia, but also in all other disorders which can be shown by modern tests to cause vitamin-B_{12} deficiency. The objects of the present paper are (1) to review critically the literature relating to the occurrence of SCD in such disorders, and (2) to describe two cases, which are unique in that the megaloblastic anaemia, due to intestinal disease, was proved by histological examination to be associated with spinal cord degeneration.

I. Review of the Literature

A. *Vitamin-B_{12} deficiency due to dietary deficiency.* Nutritional megaloblastic anaemia due to dietary deficiency of vitamin B_{12} and folic acid has been reported from many tropical and subtropical countries since the original report of Dr. Lucy Wills in Calcutta in 1931. Since the missing factor largely responsible for the development of megaloblastic anaemia in this condition is folic acid, it is not surprising that reports on the occurrence of SCD are notable by their absence. Where, however, there is a deficient intake of vitamin B_{12} for long periods, neurological manifestations would be expected to occur, and such indeed is the case. Wokes (1956) has reported the development of vitamin-B_{12} deficiency in a group of people termed 'vegans', who exclude from their diet all food from animal sources. It is rare for them to develop megaloblastic anaemia, or even macrocytosis, probably because their intake of folic acid is adequate. After several years on such a strict diet, however, the level of vitamin B_{12} in the serum may become very low, and Wokes stated that marked clinical changes, which in some instances were typical of SCD, had been found on examination of the nervous systems of 'vegans'. No clinical details were given, but the author referred to an account of such a case, probably the first of its kind, by Badenoch (1954). Badenoch investigated a boy of 19 years, a strict

vegetarian, who had the classical signs of SCD. Gastric biopsy revealed a normal gastric mucosa, and absorption of radioactive vitamin B_{12} was not impaired. The boy responded strikingly to vitamin-B_{12} therapy alone.

B. *Vitamin-B_{12} deficiency due to loss of intrinsic factor consequent on gastric operations and damage to gastric mucosa.* Dennig (1929) found macrocytic anaemia with paraesthesiae, hyperaesthesiae, ataxia, and absent knee-jerks, in a man who had had a total resection of the stomach for gastric ulcer nine years previously. Autopsy revealed degeneration of the posterior columns of the spinal cord. Rowlands and Simpson (1932) observed a similar patient who developed ataxia, loss of vibration sense in the lower limbs, loss of knee- and ankle-jerks, and bilateral extensor plantar reflexes, 15 years after partial gastrectomy for cancer of the stomach stated to be histologically proved. There appears to be only one account of SCD following gastroenterostomy. Naish and Capper (1953) demonstrated macrocytic anaemia in a man 26 years after this operation. Subsequently he received intermittent and inadequate liver therapy, and after five years he returned with well-marked peripheral neuritis. Three years later he had developed extensor plantar reflexes, and after a further year he died. Histological examination of the cord revealed degeneration of the posterior columns. In addition, at autopsy, the patient was found to have a long afferent loop proximal to the gastrojejunal anastomosis, and a large carcinoma of the stoma. It is therefore not clear whether macrocytic anaemia resulted from stagnation in the afferent loop, or from lack of intrinsic factor due to gastritis and neoplasm spreading from the stoma. It was stated, however, that those parts of the stomach not affected by the tumour had a normal histological appearance. The first description of SCD and megaloblastic anaemia associated with gastric cancer has been attributed to Waterfield and Shackle (1923). These authors gave details of a patient who had the typical clinical and histological features of SCD. Simpson (1931) pointed out that there was an earlier account of a similar case by Brandes (1921). Woltman and Heck (1937), in their very extensive review of the literature, found that examples of SCD had been attributed to chronic alcoholism. If such cases are to be accepted, it is conceivable that they had resulted from loss of intrinsic factor secondary to chronic gastritis.

C. *Vitamin-B_{12} deficiency due to intestinal disorders.* Utilization of vitamin B_{12} by parasites is a common cause of megaloblastic anaemia in some parts of the world, particularly Scandinavia, and it is of interest that, among the earliest accounts of SCD, Minnich (1892) recorded a case associated with fish-tape-worm infestation. Many similar cases have since been described (Woltman and Heck, 1937). Apart from this special group, the intestinal diseases which give rise to vitamin-B_{12} deficiency may be classified as follows:

1. *Structural changes* leading to (*a*) diversion of vitamin B_{12} by bacteria in stagnant areas of bowel due to strictures, fistulae, anastomoses, and small-intestinal diverticula; (*b*) malabsorption of vitamin B_{12} due to short-circuit operations, or to infiltration of the small bowel with tuberculosis or tumour.

After the original description by Faber in 1897 of the association of macrocytic anaemia and disorders of the intestine, Barker and Hummel (1939) were able to collect from the literature 49 cases of macrocytic anaemia associated with intestinal strictures and anastomoses. They added two cases from their own experience. Six of this group of patients complained of paraesthesiae in the extremities, but showed no objective neurological changes. In addition, one patient described by Fairley and Kilner (1931) had clinical signs of severe peripheral neuritis, and at post-mortem examination showed no evidence of spinal cord involvement, and these cases were stated to have presented features of SCD; but, after studying the original reports, we feel that two of the latter should be excluded. In the first, described by Little, Zerfas, and Trusler (1929), the patient merely had numbness and tingling of both hands and feet, without any abnormal physical signs, and in the second (Taylor, 1930) no reference was made to the nervous system. The remaining case was recorded by Hurst (1933). He observed the development of macrocytic anaemia, paraesthesiae, and bilateral extensor plantar reflexes, in a young woman, five years after two short-circuit operations for intestinal obstruction due to adhesions following appendicectomy. There was free hydrochloric acid in the gastric juice. At laparotomy the ileum and ascending colon were found to be markedly dilated, without evidence of obstruction. The patient died shortly afterwards, but no post-mortem examination was undertaken. Cameron, Watson, and Witts (1949) increased the number of reported cases of macrocytic anaemia in this group of structural diseases of the intestine to 61. They stated that clinical signs of neurological disease had been found in 12, were absent in 19, and were not recorded in 30, but no further details were given. Halsted, Swenseid, Lewis, and Gasster (1956) and Halsted, Lewis, and Gasster (1956) brought the total to 79, and stated that SCD had occurred in several cases of this type, but gave no details. We have been able to find only four clinical reports, in addition to that of Hurst mentioned above, in which neurological signs compatible with SCD were associated with intestinal disease. The first case was reported by Krevans, Conley, and Sachs (1954). They described briefly a patient who had a megaloblastic anaemia, normal gastric acidity, SCD, and multiple diverticula of the jejunum. In this patient the authors were able to demonstrate, using radioactive-cobalt-labelled cyanocobalamin, marked impairment of absorption of vitamin B_{12}, which became normal during the administration of chlortetracycline. The second case, described by Badenoch, Bedford, and Evans (1955), was that of a housewife, aged 49 years, who developed megaloblastic anaemia due to extensive jejunal diverticulosis. The most striking physical signs were in the nervous system, namely severe flaccid weakness, sensory loss of 'stocking' distribution, and absent deep reflexes in the lower limbs. The plantar responses were flexor. Post-mortem examination was undertaken, but the significance of the findings in the nervous system was not discussed (see Addendum). The third case was reported by Wilkinson (1955), who described the development of megaloblastic anaemia and the classical picture of SCD in a young woman who had a spontaneous ileocolic fistula due to duodenal

ulceration. Free hydrochloric acid was present in the gastric juice. The anaemia was corrected by parenteral liver therapy, and eventually cured by operative repair of the fistula. There was, however, only partial recovery from the neurological disease. It is interesting to note that this patient's mother died of pernicious anaemia. The fourth case was reported by Halsted, Swenseid, Lewis, and Gasster (1956), and Halsted, Lewis, and Gasster (1956). A boy aged 19 years presented megaloblastic anaemia and signs of SCD three years after the surgical treatment of intestinal obstruction. He had free hydrochloric acid in the gastric juice. With the use of ^{60}Co-labelled vitamin B_{12} malabsorption of vitamin B_{12} was demonstrated; it could be corrected by the administration of tetracycline, but not of intrinsic factor. The patient made a good recovery with parenteral vitamin B_{12} therapy.

2. *Functional disorders of the small intestine* leading to a failure in absorption of vitamin B_{12}: for example, coeliac disease, tropical sprue, and idiopathic steatorrhoea. So far as we are aware, SCD has never been described in association with coeliac disease. This is not surprising, since megaloblastic anaemia occurs very rarely in this condition. No reports have been found of the occurrence of definite SCD in tropical sprue. Fairley (1934) stated that he had 'never observed subacute combined degeneration in tropical sprue, though neuritic complications are common enough'. Stefanini (1948) reviewed 1,069 cases of tropical sprue in Italian prisoners of war in India, and found that '17 per cent. showed paraesthesiae, a few patients showed exaggerated tendon-reflexes, but as a rule the neurological examination was negative'. On the other hand Snell (1938) discovered three cases of SCD in his own series of 32 patients suffering from idiopathic steatorrhoea, and Cooke (1952) has encountered two examples in his group of 127 patients.

Summary. It is clear, therefore, from a study of the literature, that clinical features compatible with the diagnosis of SCD may develop in vitamin-B_{12} deficiency states other than Addisonian pernicious anaemia. Particular attention has been paid to a study of reports relating to the neurological features which may occur in diseases of the intestinal tract, and only 10 examples of SCD have been found, five due to structural disorders of the intestine, and five to idiopathic steatorrhoea. In only one of these cases has histological proof of SCD been obtained.

II. Report of Cases

In the present paper we describe two cases in which spinal cord disease, confirmed by histological examination, was associated with megaloblastic anaemia, due in the first patient to tuberculous disease of the small intestine, and in the second to idiopathic steatorrhoea. Brief reference has been made to the first case on a previous occasion (Davidson, 1953) and it is now presented in more detail.

Case 1. A fireman in the Merchant Navy, aged 43 years, came under observation in July 1947, when he was admitted to hospital for investigation of

diarrhoea. He had served at sea since adolescence, always in the North and South Atlantic, the Mediterranean, or the Persian Gulf. Apart from meningitis in 1942, and a herniotomy in 1945, his previous health had been excellent. In August 1946, while at sea, he started to experience diarrhoea, which persisted intermittently during the subsequent year. When first seen he was having three to five bowel motions daily, the stools being pale, semi-solid, and bulky. He complained in addition of occasional nausea and vomiting after meals, and of abdominal swelling, sometimes accompanied by pain in the lower abdomen, especially towards the end of the day. The tongue had been sore, and he had been troubled by a 'burning' sensation in the soles of the feet.

At this time the principal findings on examination were well-marked angular stomatitis and atrophic glossitis (Plate 52, Fig. 1), abdominal distension, and tenderness in both iliac fossae. The spleen was not palpable, but the liver was enlarged 3 cm. below the right costal margin. It was observed that the right abdominal reflexes were absent, the knee- and ankle-jerks were very brisk on both sides, and both plantar reflexes were extensor. There was muscle hyperalgesia in the right calf, with cutaneous hypoaesthesia of 'stocking' distribution in the lower two-thirds of the right leg. Vibration sense was absent in the left lower limb to the level of the anterior iliac spines. The findings on examination of the peripheral blood were as follows: haemoglobin 12·7 g. per 100 ml.; red cells 3,630,000 per cu. mm.; colour index 1·2; packed cell volume 37 per cent.; mean corpuscular volume 104 cu. μ; mean corpuscular haemoglobin concentration 34·2 per cent. Megaloblasts were present in smears made from bone-marrow aspirate. Hydrochloric acid was present in the gastric juice. Repeated examination of the stools failed to demonstrate any pathogenic organisms. The fat-content of the stools was greatly increased, namely 35·8 per cent. of the dry specimen; 71·3 per cent. was 'split' and 28·7 per cent. 'unsplit'. A diagnosis of tropical sprue was made, and a low-fat, high-protein diet, supplemented with iron, folic acid, and parenteral liver, was prescribed. On this régime the anaemia was corrected, but there was no lessening of diarrhoea. Although no improvement in the neurological features occurred, there was no deterioration.

In March 1948 the patient developed subacute intestinal obstruction, and laparotomy was undertaken. At operation the parietal peritoneum was found to be studded with tubercles, and in the abdominal cavity there were many masses of enlarged, matted glands. Biopsy of one of these glands revealed active tuberculosis, and it was now clear that the correct diagnosis was a malabsorption syndrome due to tuberculous disease of the small intestine. At this stage the patient showed clubbing of fingers and toes (Plate 52, Figs. 2 and 3). Objective signs of spinal cord disease, similar to those obtained previously, were clearly present. X-ray examination of the chest revealed long-standing tuberculous lesions, and tubercle bacilli were found in the sputum. Hydrochloric acid was again demonstrated in the gastric juice, and steatorrhoea was confirmed, fat-absorption being only 78 per cent. in a balance test. Although the haemoglobin in the peripheral blood was 14 to 14·8 g. per 100 ml., the red cells still showed macrocytosis. The plasma proteins were reduced (5·3 g. per 100 ml.; albumin 1·6 g., globulin 3·7 g.). The blood-calcium level was normal. In spite of intensive therapy with parenteral liver extracts, protein hydrolysates, and vitamin supplements, the patient's condition gradually deteriorated, and he died in November 1948. Immediately after death formalin was injected into the stomach by tube and into the peritoneal cavity.

At autopsy the following were the salient findings. The naked-eye and microscopic appearances of the stomach were entirely normal (Plate 53, Fig. 4). The mesenteric lymph-nodes were greatly enlarged, and section revealed

extensive active tuberculosis. The upper four or five feet of jejunum were greatly dilated, and the serous surface was thickly studded with tubercles (Plate 53, Fig. 5). On opening the gut numerous large tuberculous ulcers were found, many completely encircling the lumen. There was no stenosis. The remainder of the small and large intestine was normal. Both lungs showed tuberculous lesions at the apices. Erythropoiesis, in sections of bone-marrow, was normoblastic. In the central nervous system no abnormality was found in the leptomeninges, brain, brain-stem, or cerebellum. In the spinal cord there was, at all levels, demyelinization of the posterior columns, the columns of Goll only being affected (Plate 53, Fig. 6). The changes were most marked in the lower dorsal and lumbar segment, but were also evident in the cervical region. There was a mild degree of involvement of the lateral columns. In the damaged columns there was little evidence of replacement gliosis. The appearances taken as a whole were considered by the neuropathologist to be in keeping with subacute combined degeneration of the cord.

Final diagnosis. 1. Megaloblastic anaemia (corrected) and subacute combined degeneration of the spinal cord due to intestinal malabsorption. 2. Tuberculous enteritis, mesenteric adenitis, and peritonitis. 3. Pulmonary tuberculosis.

Case 2. A man employed in the Army and Civil Service, aged 45 years at the time of his admission. This patient was in the Army throughout the 1939–45 war. His overseas service was spent in North Africa and Italy. He was in good health until January 1944, when he began to experience loss of appetite and vague abdominal pains. In January 1945 he 'reported sick' because of increasing fatigue, breathlessness on exertion, and episodes of constipation and diarrhoea. A provisional diagnosis of aplastic anaemia was made. In March 1945 the haematological features were reported as being typical of pernicious anaemia, and appropriate treatment was given. The patient was discharged from the Army with this diagnosis in December 1945. In November 1945, however, there was evidence in his Army records that, despite weekly injections of liver extract, the blood picture was not satisfactory: haemoglobin 15·7 g. per 100 ml.; red cells 3,570,000 per cu. mm.; colour index 1·51.

In 1946 he was investigated in the Royal Infirmary, Edinburgh, and considered to have tropical sprue. Folic acid and injections of liver extract were prescribed, and he remained well until his treatment was stopped in another hospital in October 1947. Thereafter there was a gradual return of fatigue, abdominal pains, and diarrhoea, and in August 1948 he was admitted to a hospital in England because of these symptoms. Folic-acid therapy was resumed. He improved, and remained in fairly good health till 1952. In March of that year he felt unduly tired, and in August he was readmitted to the Royal Infirmary, Edinburgh, primarily because for three months he had been troubled by weakness in the legs and paraesthesiae in the hands and forearms. In addition he complained of abdominal distension and flatulence, and was passing several loose porridge-like stools each day. At this time there was generalized weakness of muscle power, and the knee- and ankle-jerks could not be elicited. No other abnormalities were demonstrated in the nervous system. Subsequently he received continuous treatment with folic acid, cyanocobalamin, and dietary supplements of calcium lactate, ascorbic acid, and a vitamin-B-complex preparation. Latent tetany had been considered as a possible explanation for the paraesthesiae, in view of the frequent finding of low levels of calcium in the blood. This view was supported by an episode of overt tetany in 1953, but at no time did the parenteral or oral administration of calcium relieve the paraesthesiae. From July 1953 the patient was fit for full-time employment in an office,

but continued to have variable diarrhoea with three to six pale, bulky motions daily.

His final admission to hospital, in January 1956, was occasioned by progressive lassitude and loss of weight for about 12 months. He was found to be emaciated (weight 6 stone 12 lb.), and showed striking clubbing of the fingers. There was slight atrophy of the lingual papillae. The abdomen was swollen owing to gaseous distension of the bowel. There had been no increase in his neurological symptoms since 1952. Examination of the nervous system revealed the following abnormalities: generalized weakness of muscle power; slight impairment of touch sensation, of a 'glove' distribution, in the hands; in the lower limbs slight impairment of vibration, touch, and pain sensation, in a 'stocking' distribution, and of positional sensation; absence of deep reflexes in all four limbs. The abdominal reflexes were present, and the plantar responses were flexor. General physical examination otherwise gave negative results.

The investigations which were undertaken while the patient was under observation in Edinburgh may be summarized as follows:

Peripheral blood findings	Normal since 1950.
Gastric analysis (March 1953)	No free hydrochloric acid after 0·5 mg. of histamine.
Fat-content of stool (February 1952)	Gross malabsorption. Mean daily output of fat 36·9 g. (normal < 6 g.).
Oral glucose-tolerance test (May 1954)	Flat curve. Maximum rise 11 mg. per 100 ml. after one and a half hours.
X-ray findings:	
Chest (May 1953 to January 1956)	Negative.
Barium meal and follow-through	Marked flocculation of barium in the small intestine.
Liver function tests (September 1952)	Normal.
Differential urinary folic-acid absorption and excretion test	Gross malabsorption of folic acid.
Absorption of ^{56}Co-labelled vitamin B_{12}	Moderate impairment of absorption.

In view of the grave state of the patient and his failure to remain well while receiving folic acid, vitamin B_{12}, and many dietary supplements, it was decided to administer cortisone. On steroid therapy there was a transient improvement in the frequency of diarrhoea, lasting a few days, but thereafter the patient rapidly deteriorated, and died in February 1956.

At autopsy the cause of death was found to be tuberculous and pyogenic bronchopneumonia. Since no abnormality had been seen on radiological examination of the chest one month before death, and since there was no evidence of chronic tuberculous disease, the tuberculous lesion was believed to be a terminal event due to activation of an old focus. In the stomach there were multiple acute ulcers, and the sections showed evidence of atrophic gastritis (Plate 54, Fig. 7). There was no evidence of tuberculosis or any other specific disease in the small intestine. Examination of the central nervous system revealed no significant abnormality of the leptomeninges, brain, brain-stem, or cerebellum. In the spinal cord there was marked atrophy of the posterior columns at all levels (Plate 54, Fig. 8). In these columns there was loss of axis cylinders and myelin sheaths, with a concomitant gliosis. No inflammatory changes were seen. The anterior and lateral columns and the central grey columns were normal. The nerve-roots, especially the posterior

roots, showed slight but definite demyelinization and mild endoneural fibrosis. In sections of the ulnar nerve and sciatic nerve patchy changes of a similar nature were to be seen.

Final diagnosis. Idiopathic steatorrhoea leading to malabsorption of vitamin B_{12}, folic acid, calcium, glucose, and fat. The macrocytic anaemia, and the histological changes in the peripheral nerves and posterior columns of the spinal cord, were presumably determined by deficiency of vitamin B_{12}, which was shown to be present during life.

Summary. In the two cases which have been described, macrocytic anaemia (known to be due in the first case to megaloblastic blood formation) was associated with an intestinal disorder. Histological examination of the spinal cords obtained at autopsy revealed features compatible with the diagnosis of SCD, namely demyelinization in the first case of the posterior and lateral columns, and in the second case of the posterior columns. In neither case were the histological appearances typical of the descriptions of advanced SCD as given in the literature. The changes in Case 2 were very similar to the ascending degeneration which follows injury to the cells in the posterior root ganglia. It is also pertinent that patients with intestinal disease may have malabsorption of factors other than vitamin B_{12}, both known and unknown, deficiency of which might give rise to spinal cord disease, for example thiamine or nicotinic acid. It should be emphasized, however, that in both patients the neurological picture did not deteriorate after the institution of vitamin-B_{12} therapy administered parenterally in the form of highly purified liver extract ('anahaemin'). It is also significant that in the second case the neurological symptoms developed while the patient was receiving treatment with folic acid alone. Moreover, in this patient malabsorption of radioactive-cobalt-labelled vitamin B_{12} was demonstrated. The histological appearances of early SCD are not known, and opportunity seldom arises to study the spinal cord from a patient who has received treatment for a long period and in whom the disease has been arrested. We believe, after considering all the factors, that these two patients were suffering from SCD.

Discussion

Although in the great majority of cases SCD is associated with Addisonian pernicious anaemia, we have shown, from a review of the literature and from the cases described above, that other conditions which give rise to vitamin-B_{12} deficiency may rarely cause the same neurological syndrome. In such cases free hydrochloric acid may be present in the gastric juice. Hence, contrary to the view expressed in several major neurological textbooks (Brain, 1955; Wilson, 1955; Purves-Stewart and Worster-Drought, 1952; Walshe, 1955), achlorhydria is not necessarily a constant feature in SCD any more than it is of non-Addisonian megaloblastic anaemia.

The mechanism by which lack of vitamin B_{12} gives rise to subacute combined degeneration of the spinal cord in man has not been established, and at least three interesting observations have still to be explained. First, there is little

association between the effects of vitamin-B_{12} deficiency on the blood and its effects on the nervous system. Patients with Addisonian pernicious anaemia may present a profound anaemia and no abnormal neurological features. On the other hand, as is well known, the manifestations of SCD may rarely precede the development of anaemia by long periods. If, as is believed, vitamin B_{12} is necessary for nucleic-acid synthesis, it can be understood why the blood-forming organs are affected before the nervous system, since in the former there is intense mitotic activity. It is not easy to explain how the reverse can occasionally happen.

Secondly, it might be expected that prior to the discovery of liver therapy, when patients with Addisonian pernicious anaemia were dying as the result of vitamin-B_{12} deficiency, the incidence of SCD would be very much higher than it is today. It appears that this was not the case. The incidence of SCD in Addisonian anaemia recorded in the literature of that period varied between 2·8 per cent. (Bramwell, 1915) and 80 per cent. (Woltman, 1919). When rigid criteria were applied to the differentiation of peripheral neuritis from cord disease, several writers found that the incidence of SCD was low; for example, Cabot (1908) reported 11 per cent. of 1,200 cases, Hunter (1925) 9 per cent. of 200 cases; and in the experience of Davidson and Gulland (1930) the incidence was 5 per cent. The incidence of SCD which these authors report is very similar to that found by Davidson (1957), who observed cord disease in 7 per cent. of 250 cases of Addisonian pernicious anaemia investigated since 1944. It is interesting to speculate why the incidence of SCD, as quoted in these series, does not appear to have been markedly altered by the discovery of liver therapy. Before 1926 most persons with Addisonian pernicious anaemia died as the result of anaemia, presumably before cord disease had time to develop. Since 1926 there can be no doubt that some patients received inadequate liver therapy. In such patients the haemoglobin concentration and red-cell count was maintained at subnormal levels for many years and, although during this time life was sustained and ordinary activities were possible, the nervous system was submitted to a chronic insufficiency of a necessary metabolic factor. Accordingly such patients were potential candidates for SCD.

Thirdly, folic-acid deficiency gives rise to a megaloblastic anaemia indistinguishable from that of vitamin-B_{12} deficiency, but does not cause neurological disease. In addition, although in most patients folic acid will correct Addisonian pernicious anaemia, it has no beneficial effect on and may indeed aggravate SCD.

The precise biochemical functions of folic acid and vitamin B_{12} are imperfectly understood. Some hypotheses which might explain the different action of these two substances on the nervous system have been discussed by Smith (1956) and Sinclair (1956). Nieweg, Faber, de Vries, and Kroese (1954) put forward a theory to explain why the long fibre tracts of the spinal cord are predominantly affected in SCD, and Alexander (1957) has given an account of the effect of experimental vitamin-B_{12} deficiency on the nervous system of animals. The fact that some persons develop SCD before the blood is affected, while others show no evidence of neurological disease after protracted and severe anaemia,

might be explained on the hypothesis that there is great individual variation in the susceptibility of the nervous system to vitamin-B_{12} deficiency. Alternatively, it might be justifiable to revive an old theory that a second substance (neuropoietic factor), in addition to vitamin B_{12} but closely related to it, is necessary for the integrity of the nervous system. In such circumstances the severity of deficiency of the neuropoietic factor relative to vitamin B_{12} could determine at what stage of the patient's illness SCD will develop.

Most of the knowledge regarding the pathogenesis of the non-Addisonian megaloblastic anaemias has been acquired in recent years, and these conditions are rare compared with Addisonian pernicious anaemia. From a study of 1,000 cases seen in Edinburgh it can be said that in Britain, if a patient has a macrocytic anaemia, it is seven chances to one that he is suffering from Addisonian pernicious anaemia. The remaining cases of megaloblastic anaemia in the Edinburgh series were due mainly to pregnancy (instances which, being attributable to a lack of folic acid, are not associated with SCD) (90 cases), and to diseases of the intestinal tract (49 cases). Less than 10 per cent. of patients with Addisonian pernicious anaemia suffer from SCD, and there is no doubt that in the other megaloblastic anaemias due to vitamin-B_{12} deficiency the incidence of spinal cord disease is very much lower, possibly one per cent. or less. In a review of published records in which SCD has been claimed to be associated with a *structural or functional disorder of the intestine*, we have been able to accept the reported evidence in only 10 cases. In the present paper two further cases have been described. So far as we are aware, these two, and the one described by Badenoch, Bedford, and Evans (1955), are the only cases in which the presence of SCD has been confirmed by histological examination. It seems to us to be important to establish in all obscure megaloblastic anaemias whether there is deficiency of folic acid or vitamin B_{12}, or both. This is because the administration of folic acid will correct most megaloblastic anaemias, at least for a time. Hence vitamin-B_{12} deficiency may pass unrecognized, and in such circumstances the patient may develop SCD.

Russell, Batten, and Collier (1900) were the first of many authors to criticize the term 'subacute combined degeneration of the spinal cord'. We suggest that the name should be discarded for the following reasons: first, the disease usually begins insidiously and not subacutely; secondly, the combination of lesions in the posterior and lateral tracts is frequently absent; thirdly, the name is cumbersome; and finally, it does not take into account the fact that peripheral neuritis develops first and is almost always associated with degeneration in the cord, and that in a small proportion of cases there are mental changes due to brain damage. Accordingly, we suggest that the title 'vitamin B_{12} neuropathy' should be used in preference to 'subacute combined degeneration of the cord' to describe the neurological manifestations of vitamin-B_{12} deficiency. We would justify the use of the term neuropathy in a comprehensive sense, to cover damage to any part of the nervous system, rather than in the restrictive sense to damage solely to the peripheral nerves, by reference to its definition in such works as the *Oxford English Dictionary* and Gould's *Medical Dictionary*.

We wish to thank Dr. A. F. J. Maloney for the histological reports on the spinal cord in the two cases described in the paper.

ADDENDUM

We are grateful to Professor L. J. Witts and Dr. D. Oppenheimer, of the Radcliffe Infirmary, Oxford, for letting us examine the sections of the nervous system in the case of megaloblastic anaemia due to extensive diverticulosis of jejunum reported by Badenoch, Bedford, and Evans (1955), and for giving us permission to record the main pathological findings, which were described by Dr. Oppenheimer as follows:

No significant changes were observed in the cerebrum or brain-stem. In the cerebellum there was slight demyelinization of the central white matter, with scattered loss of Purkinje and granule cells. In the spinal cord there were scattered foci of 'spongy' demyelinization at various levels, but particularly in the lumbar segment of the cord (Plate 55, Fig. 9), without necrosis, or inflammatory or glial reaction. The foci were to be seen mainly in the periphery of the cord, and did not correspond to anatomical tracts. Haemorrhages were found in several posterior root ganglia, and groups of inflammatory cells were observed in their dural sheaths. In the anterior and posterior sacral roots, and in the right and left ulnar and median nerves, there were a few degenerating fibres. The changes in the nervous system were considered by Dr. Oppenheimer, and by Dr. Maloney, Neuropathologist at the Royal Infirmary, Edinburgh, to be compatible with SCD. No explanation for the high level of protein in the cerebrospinal fluid prior to the patient's death is available. According to M. Locoge and J. N. Cumings (1958; *Brit med. J.* **1**, 618) the protein content may be raised in approximately 50 per cent. of patients with SCD. Only on rare occasions, however, does the level exceed 100 mg. per 100 ml.

This case is therefore the third in which subacute combined degeneration of the spinal cord, confirmed by post-mortem examination, was already associated with megaloblastic anaemia due to intestinal disease.

Summary

1. The literature dealing with subacute combined degeneration of the spinal cord (SCD) is reviewed. The clinical and pathological features of this condition are briefly described. Particular reference is made to its occurrence in non-Addisonian megaloblastic anaemia.

2. Two cases are described in which macrocytic anaemia and SCD were due to intestinal malabsorption. Ten other cases of SCD associated with structural or functional disorders of the intestine have been recorded, but the two cases reported in the present paper, and the case reported in the Addendum, appear to be the only ones in which the clinical diagnosis of cord disease has been confirmed by histological examination.

3. It is suggested that the term 'vitamin B_{12} neuropathy' is preferable to 'subacute combined degeneration of the cord' as a title for the neurological manifestations of vitamin-B_{12} deficiency.

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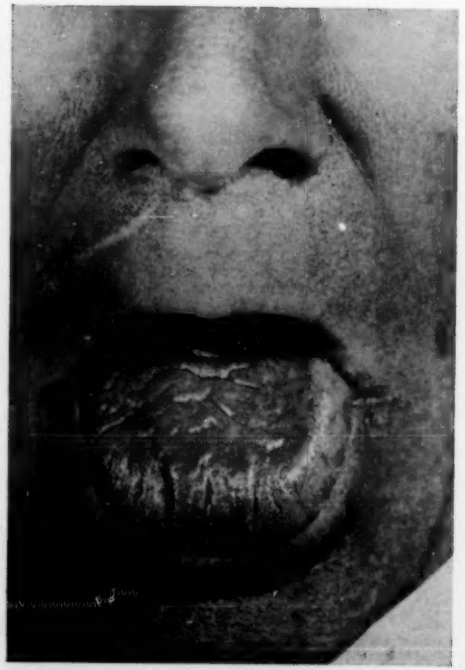

FIG. 1. Case 1. Atrophic glossitis and angular stomatitis

FIG. 2. Case 1. Severe clubbing of the fingers

FIG. 3. Case 1. Clubbing of the toes

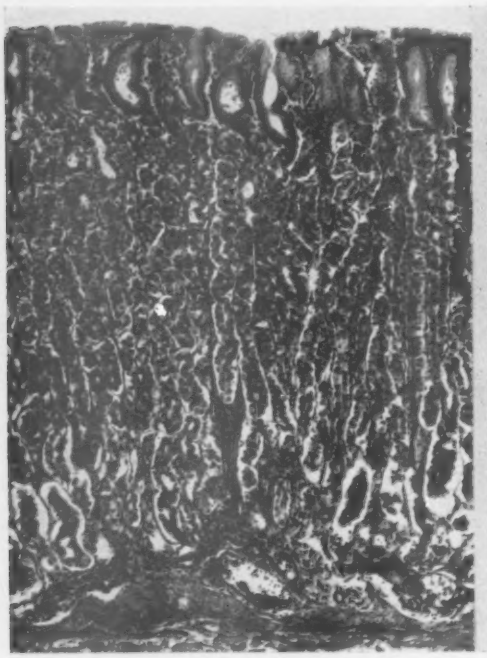

FIG. 4. Case 1. Normal mucosa from the body of the stomach (haematoxylin and eosin, ×70)

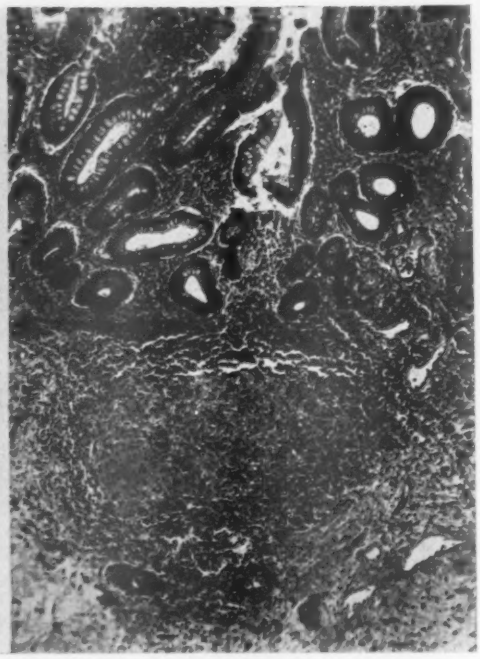

FIG. 5. Case 1. Tubercule in the submucosa of the jejunum (haematoxylin and eosin, ×75)

FIG. 6. Case 1. Spinal cord at the lower thoracic level, showing severe demyelinization in the columns of Goll and slight demyelization at the periphery of the columns of Burdach and the lateral columns (Smith and Quigley's stain, ×10)

FIG. 7. Case 2. Mucosa from the body of the stomach, showing atrophy and infiltration with chronic inflammatory cells (haematoxylin and eosin, ×70)

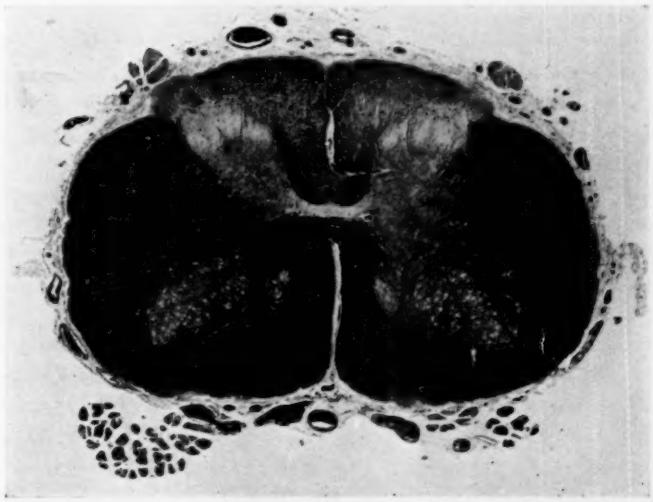

FIG. 8. Case 2. Spinal cord at the lumbar level, showing severe demyelinization in the posterior columns and in the posterior nerve-roots (Weigert-Pal stain, ×10)

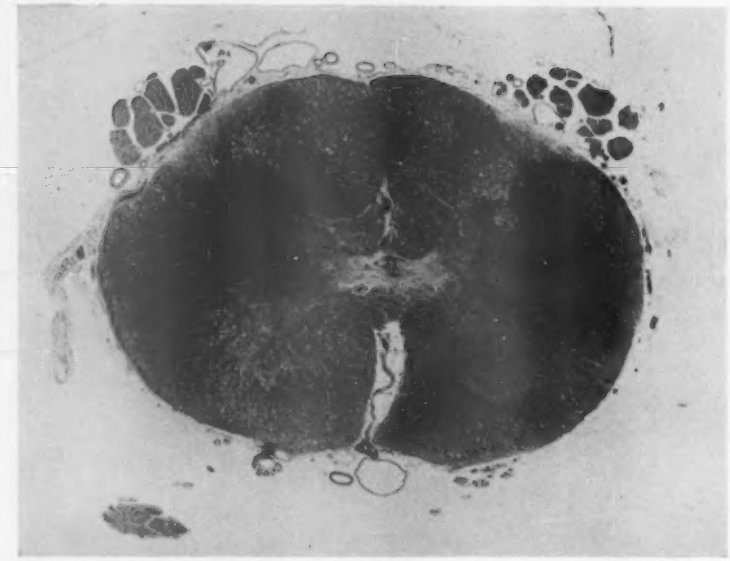

FIG. 9. Spinal cord at the lumbar level from the case of jejunal diverticulosis with megaloblastic anaemia and SCD recorded by Badenoch, Bedford, and Evans (1955) and reported in the Addendum (Weigert-Pal stain, $\times 10$)

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SERUM TRANSAMINASE ESTIMATION IN THE INVESTIGATION OF MYOCARDIAL INFARCTION[1]

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TRANSAMINATING enzymes play a fundamental role in the nitrogenous metabolism of living tissues. They catalyse the reversible exchange of the α-amino group of an L-amino acid for the α-keto group of a keto acid to form a new amino acid and a new keto acid, the reaction requiring pyridoxal phosphate as coenzyme:

$$R.CH.NH_2.COOH + R'.CO.COOH \xrightleftharpoons{\text{Transaminase}} R.CO.COOH + R'.CH.NH_2.COOH.$$

Most of the naturally occurring amino acids take part in these reactions, each of which is catalysed by a specific transaminase. Transaminases are found in bacteria, plants, and animals. In animal tissues the predominant transaminases are glutamic-oxaloacetic (G-OT) and glutamic-pyruvic (G-PT), which catalyse reactions (1) and (2) respectively:

(1) L-glutamate + oxaloacetate ⇌ L-aspartate + α-oxoglutarate.
(2) L-glutamate + pyruvate ⇌ L-alanine + α-oxoglutarate.

These enzymes are unevenly distributed in body tissues. G-OT is present in highest concentration in heart muscle (Cohen, 1940), followed by skeletal muscle, liver, brain, and kidney, while G-PT occurs in greatest concentration in liver, but in low concentration in heart and skeletal muscle (Awapara and Seale, 1952; Wróblewski and LaDue, 1956). Because of this our studies of serum transaminases in myocardial disease have been confined to G-OT, which we shall call simply transaminase (Baron, Bell, and Oakley, 1956). Many other serum enzymes have since been investigated for their diagnostic value in myocardial infarction, but they will not be considered here, and except for lactic dehydrogenase (Wróblewski and LaDue, 1955; Baron, Bell, and Oakley, 1957) they are at present of interest only to research workers. LaDue, Wróblewski, and Karmen (1954) first showed that a great increase in glutamic oxaloacetic transaminase in the serum follows myocardial infarction in man. Experimental work followed, and in dogs subjected to coronary occlusion an increase of serum transaminase was found which was directly correlated with the amount of infarction estimated at autopsy (Agress, Jacobs, Glassner, Lederer, Clark, Wróblewski, Karmen, and LaDue, 1955). The theoretical and experimental justification for estimation of

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G-OT in myocardial infarction was thus established. There have been many reports on the mechanism and diagnostic value of changes in transaminase and in other serum enzymes in disease, especially in myocardial and hepatic disorders. The object of the present study was to assess the value of estimations of serum transaminase in the diagnosis and prognosis of myocardial infarction, and their practical use to the clinician.

Patients Investigated

Serum transaminase activity was estimated in a series of 170 patients between February 1956 and June 1957. They included patients in the wards and outpatients at the Royal Free Hospital, and patients from other hospitals. The investigation was done in cases of suspected myocardial infarction, as well as in various other conditions, particularly those which present problems of diagnosis from myocardial infarction. The diagnoses were based on clinical assessment with electrocardiographic evidence and other laboratory investigations. Postmortem confirmation was obtained in 19 cases. Some specimens were collected at fixed times after the onset of the disorder, but the majority were taken at random, the time of collection being noted. Sera were obtained from healthy volunteers for establishment of the normal range.

TABLE I

Temperature Correction

Correction factors to be employed to convert serum-transaminase results at different working temperatures to activity at 20° C. This table was constructed from a graph made by measuring the transaminase activities of the same sera at different temperatures.

Working temperature (°C)	*Correction factors*
16	1·47
18	1·19
20	1·0
22	0·87
24	0·77
26	0·69

Methods

The standard method of Karmen (1955), using a Unicam SP 500 spectrophotometer, was employed for all transaminase analyses reported in the present paper. The method was modified by the use of glass cells instead of quartz cells (for cheapness), and by the use of temperature correction factors (Table I). Temperature correction was found necessary because of the variations in temperature experienced in our laboratory, and their large effect on transaminase estimations. All results were corrected to 20° C, which was our average laboratory temperature. Experiments were also carried out with the colorimetric method of Cabaud, Leeper, and Wróblewski (1956) and Reitman and Frankel (1957). The reagents for these methods are cheaper than those used for the spectrophotometric method, the techniques are simpler, and an ultra-violet spectrophotometer is not required. The method of Cabaud, Leeper, and Wróblewski

(1956) did not give reproducible results in our hands, but we found the method of Reitman and Frankel (1957) satisfactory for routine work. Tests run in parallel with the spectrophotometric method gave comparable results, except that sera which were very lipaemic, or had very high transaminase values, gave accurate results only with the spectrophotometric method. Serum for transaminase estimation could be kept without loss of activity for four days at room temperature, and at least two weeks in the 'deep freeze'. Specimens which were very much haemolysed gave falsely high results.

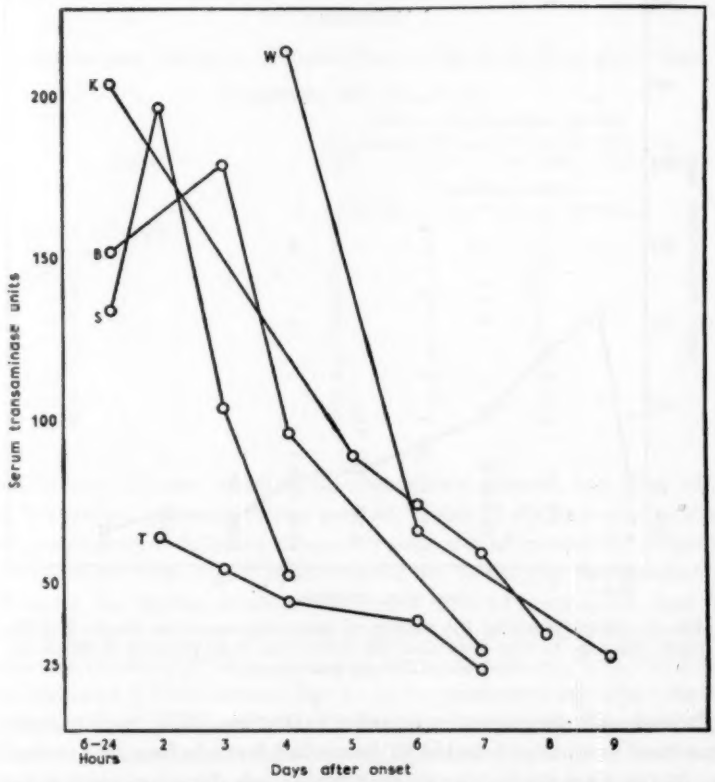

FIG. 1. Graph showing serial serum-transaminase estimations, in five patients with typical myocardial infarction, at known times after the onset.

Results

1. *Normal subjects.* The mean serum-transaminase value in 25 normal subjects was 16 units at 20° C, with a standard deviation (S.D.) of $\pm 5{\cdot}5$ units: the range was 8 to 25 units. Results not greater than 2 S.D. above the mean are considered normal (27 units or below), while results 2 and 3 S.D. above the mean are referred to as borderline (28 to 32 units). The method is very accurate: analysis of duplicates showed the standard deviation of a single estimation to be 0·6 unit.

Serum transaminase did not vary significantly from day to day, or alter with meals or exercise. This normal stability is important in assessing a rising or falling value. Application of a tourniquet for collection of blood did not affect the results.

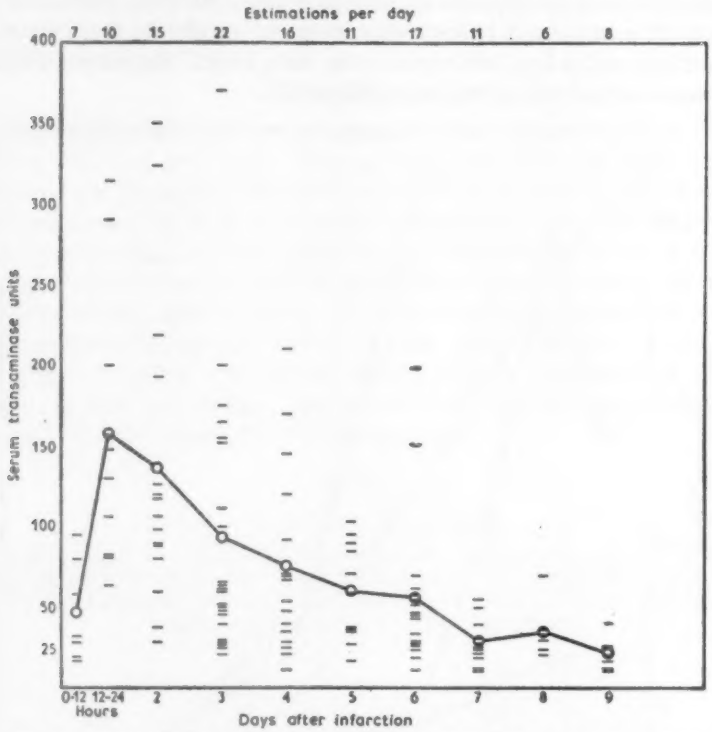

FIG. 2. Graph showing the scatter of serum-transaminase levels, and the mean value up to nine days after the infarction, in all patients in whom the time of onset was known.

2. *Patients with unequivocal myocardial infarction.* Of 71 such patients, high transaminase levels were found in 49, borderline levels in four, and normal levels in 18. In the 49 patients who showed high levels, blood specimens had been taken from two hours to nine days after the clinical onset of myocardial infarction, and the levels varied from 33 units to 1,600 units on one or more occasions. Fig. 1 shows representative graphs of five of these patients. Of the 22 patients in whom normal or borderline levels of serum transaminase were found, in 13 the blood was taken eight days or more after the clinical onset of myocardial infarction, when the transaminase has usually returned to normal. In the other nine patients single specimens only were taken, in three cases within 12 hours of the onset, which is often too early for the expected elevation. The other six specimens were taken at least three days after the onset, but the time of onset in these cases was not accurately known. In 50 patients, in whom the time of

onset of infarction was known with certainty, a composite graph of daily levels of serum transaminase in the first nine days after myocardial infarction is shown in Fig. 2. This graph shows the means of all the levels on each day after infarction, and the actual levels to show the scatter. The first 24-hour period after infarction is divided, to indicate those estimations known to have been made in the first 12 hours and those made between 12 and 24 hours after infarction. In the first 12 hours normal levels were sometimes found; in the period

TABLE II

Transaminase Values at Various Times after the Onset of Infarction

(50 patients, 123 estimations)

Time	*Serum transaminase (units)*			
	0–27	*28–50*	*51–200*	*Over 200*
	Number of cases			
Under 12 hours	2	2	3	0
12–24 hours	0	0	8	2
Day 2	0	2	10	3
„ 3	3	6	12	1
„ 4	3	4	8	1
„ 5	1	4	6	0
„ 6	4	6	7	0
„ 7	6	4	1	0
„ 8	2	3	1	0
„ 9	7	1	0	0

between 12 and 48 hours raised levels were always present, and after 48 hours normal levels were again sometimes present. Table II shows the level of serum transaminase found at different times after the onset of myocardial infarction in these 50 patients. Like Fig. 2, it shows that low levels may be expected in the first 12 hours, the highest levels in the period from 12 to 48 hours, and then a gradual fall to normal by the seventh or ninth day. A further increase in serum transaminase occurs if there is extension of the infarction. This was found in five patients, and is illustrated in Fig. 3. In five patients a secondary rise in the transaminase level was found without clinical evidence of further infarction.

3. *Patients with a provisional diagnosis of myocardial infarction.* In 10 patients the clinical diagnosis could not be confirmed because the electrocardiographic evidence, though often suggestive, was not definite, owing to extensive old changes, left bundle branch block, or certain arrhythmias. In all these patients serum-transaminase levels were raised on one or more occasions, a finding which supported the clinical diagnosis.

4. *Patients with angina of effort.* Twenty-one patients had angina of effort or acute coronary insufficiency, without clinical evidence of infarction. In 14 patients normal levels only were recorded, and in seven the serum-transaminase levels were raised, but not above 40 units.

5. *Patients with pulmonary embolism.* There were 12 such patients. Five, who were not shocked, did not have raised serum-transaminase levels. In seven

the levels were raised, and in four of these patients the pulmonary embolism occurred as a complication of myocardial infarction. One patient also had cerebral embolism and jaundice. The other two had severe shock and hypotension, which lasted several days. In neither was the level above 70 units, and this rise did not occur in the first 48 hours after the embolism.

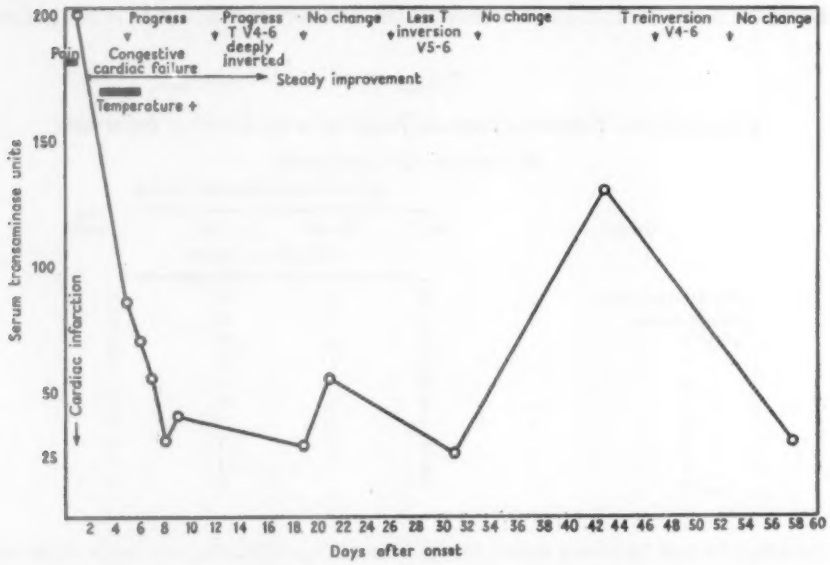

FIG. 3. Graph showing a secondary rise of the serum-transaminase level in one patient in whom an extension of infarction was shown by electrocardiographic changes.

6. *Patients with chest pain.* In the remaining group of patients, who had chest pain without electrocardiographic evidence of myocardial infarction, the serum-transaminase levels were always normal, often on repeated estimation. This group included the following: five cases of pneumonia, including bronchopneumonia, inhalation pneumonia, and primary atypical pneumonia; four cases of acute bronchitis, with or without chronic bronchitis and emphysema; three cases of functional chest pain; two cases of chronic rheumatic heart disease; and one each of duodenal ulcer, 'windy dyspepsia', oesophageal spasm in hiatus hernia, diverticulitis, cholelithiasis without jaundice, influenza, benign pericarditis, and pleural effusion.

7. *Patients with other diseases showing raised transaminase levels.* Raised transaminase levels were also found on one or more occasions in the following conditions: liver disease (acute hepatic necrosis; primary and metastatic carcinoma; obstructive jaundice; infective hepatitis; cirrhosis); acute pancreatitis; polymyositis; acute rheumatic myocarditis; heart-muscle trauma at mitral valvotomy (trauma of skeletal muscle only, at an orthopoedic operation, did not affect the serum transaminase); ruptured kidney. In three other cases slightly raised

levels (32 to 40 units) were found with no evident cause: these included two cases of bronchitis and emphysema without evidence of myocardial infarction, and one case of hypertensive heart failure with paroxysmal tachycardia, in which post-mortem examination showed no evidence of recent infarction.

Discussion

Our normal values are in agreement with those found by the great majority of other workers (Karmen, Wróblewski, and LaDue, 1955; Ostrow, Steinberg, Ticktin, and Evans, 1955; Cabaud, Leeper, and Wróblewski, 1956; Chinsky, Shmagranoff, and Sherry, 1956; Ruskin, Mahaffey, and Ruskin, 1956).

The results in our patients show that, in myocardial infarction, a single specimen of serum will always show a raised transaminase value if it is taken between 12 and 48 hours from the onset. Values above normal are generally found up to five days, and often later. A single specimen of serum taken after 48 hours may not show a diagnostically high value, but this does not necessarily exclude infarction. It is in such cases that repeated examination may be helpful, by showing a falling value and subsequent stabilization. Similarly a small infarct, causing a rise only just outside normal limits (Fig. 1, Case T) may be confirmed by finding a falling value. These results do not differ substantially from those of other workers (LaDue and Wróblewski, 1955; Ostrow, Steinberg, Ticktin, and Evans, 1955; Amelung and Horn, 1956; Biörck and Hanson, 1956; Chinsky, Shmagranoff, and Sherry, 1956; Kattus, Watanabe, Semenson, Drell, and Agress, 1956; Hanson and Biörck, 1957). In the majority of patients in whom a clinical diagnosis of myocardial infarction is made, the electrocardiogram provides rapid confirmation, and serum-transaminase estimations are not necessary for diagnosis. In the minority of cases in which the electrocardiogram is normal, or shows those changes which preclude a certain diagnosis of infarction, transaminase estimation is very useful. We have found that, in cases which show only a slight increase of serum transaminase, serial estimations may establish the diagnosis in retrospect. In the diagnosis of extension of infarction the electrocardiogram may provide immediate confirmatory evidence. When it fails to do so, transaminase estimation is valuable, as reported also by LaDue and Wróblewski (1955). Our clinical impression is that the larger the infarct, the higher is the peak and the more prolonged the rise, and that the latter findings indicate a worse prognosis. Clearly consideration only of peak values may be misleading, because the true peak is usually missed, and because all the necrosis may not occur in a single episode. The mortality seemed higher in our series when values above 300 units were found.

In angina of effort we found a small rise in transaminase values in one-third of the cases. There have been conflicting reports on this subject: Steinberg and Ostrow (1955) found only normal values; Chinsky, Shmagranoff, and Sherry (1956) found normal and borderline results; Walsh, Humoller, and Gillick (1957) found values up to 100 units. The slight elevations found in some cases of

angina of effort, and in acute coronary insufficiency, may reflect subendocardial infarction or multiple small focal necroses (Fulton, 1956).

Serum-transaminase estimation is valuable in diagnosis from other conditions. Pulmonary embolism is the only other cause of chest pain to give significant increases, and in our series such increases did not occur in uncomplicated cases. When pulmonary embolism caused prolonged shock we found a moderate rise of the transaminase level, but in myocardial infarction causing comparable shock a rise above 200 units was invariably found; in addition, the rise after pulmonary embolism is delayed. Moderate increases have been found in shock and hypotension due to other causes, without demonstrable myocardial injury (Merrill, Lemley-Stone, Grace, and Meneely, 1956), and may be due to ischaemic damage to liver cells or to impaired coronary circulation. Patchy infarction, usually in the left ventricle, has been reported in massive pulmonary embolism, causing acute cor pulmonale (Dack, Master, Horn, Grishman, and Field, 1949), and this may explain the delayed rise in transaminase levels in some cases. The conflicting reports concerning experimental and clinical pulmonary embolism can be explained by consideration of these factors. Agress, Glassner, and Jacobs (1956) and Goldstein, Israel, and Seligson (1956) have found consistently normal levels in this condition, while Denney, McAuley, Martin, Ware, and Segalove (1956) and Ostrow, Polis, and Evans (1956) reported occasional moderate increases. We have not found the consistent increase of transaminase in pulmonary disease reported by Walsh, Humoller, and Gillick (1957). Liver disease and acute pancreatitis are causes of upper abdominal pain which show a high serum-transaminase value. Hepatocellular damage is rarely a diagnostic problem, and cases of acute pancreatitis showing high serum-transaminase values have also shown high levels of serum amylase. Difficulty may arise, however (though we have not yet met such a case) in diagnosis between myocardial infarction and acute pancreatitis in a patient who has been treated with morphia, as opiates may themselves cause an increase of serum amylase (Bogoch, Roth, and Bockus, 1954).

We consider, therefore, that estimation of serum transaminase is a necessary hospital diagnostic aid in myocardial disease. It may be essential to the correct diagnosis of doubtful cases of myocardial infarction, and if a single estimation does not give a diagnostic result it should be repeated 24 hours and 48 hours later. In a typical case, with characteristic clinical and electrocardiographic findings, the estimation is unnecessary for diagnostic purposes, but may have prognostic value.

We thank Dr. Frances Gardner, Physician to the Cardiac Department, Royal Free Hospital, for her advice and criticism. We are grateful to the physicians and surgeons of the Royal Free Hospital Group, to Dr. Lawson MacDonald of the National Heart Hospital, and to Dr. Porter and Dr. Joules of the Central Middlesex Hospital, for supplying blood for analysis from patients under their care, and for access to their case notes.

Summary

1. The mean normal level of serum transaminase, at 20° C, was 16 units (S.D. $\pm$5·5 units).

2. In unequivocal myocardial infarction the serum-transaminase level was always raised 12 to 48 hours after the infarction, and usually for several more days. Extension of infarction caused a secondary increase in transaminase.

3. In angina of effort, and in pulmonary embolism with complications, moderate elevations were occasionally found. The reasons for this, and the importance in diagnosis, are discussed.

4. No significant rise was found in patients with chest pain from other causes.

5. Serum-transaminase estimation is a recommended diagnostic tool for the investigation of clinically doubtful cases of myocardial infarction, and may also have prognostic value.

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THE LOSS AND REPLACEMENT OF RED CELLS IN PATIENTS WITH ACUTE GASTROINTESTINAL HAEMORRHAGE[1]

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THE assessment of the amount of blood lost during acute gastrointestinal haemorrhage is frequently a difficult clinical problem. Such an estimate has to be made in considering the necessity for blood transfusion, and in deciding the volume to be transfused. It is probable that the tendency has been to underestimate the extent to which the red-cell volume is depleted. Extensive studies of the blood volume after trauma (Emerson and Ebert, 1945; Grant and Reeve, 1951; Prentice, Olney, Artz, and Howard, 1954; Clarke, Topley, and Flear, 1955) have revealed the magnitude of blood loss, the total blood volume being depleted by at least 50 per cent. in severe injuries. There have been few quantitative studies in acute gastrointenstinal haemorrhage. Tibbs (1956) has shown that the volume of blood lost may be very large, frequently two to four litres. In most previous studies red-cell volume has been calculated from plasma volume and the venous haematocrit. This method is not entirely satisfactory in cases of acute haemorrhage, as the ratio of body haematocrit to venous haematocrit may be abnormally low (Hope and Verel, 1955), and cannot be predicted. In assessing the loss of blood, an estimated normal red-cell volume or total blood volume has frequently been used, calculated from height and weight. The normal variation is so great (Hicks, Hope, Turnbull, and Verel, 1956) that this introduces a further inaccuracy in the estimate.

In the present study of the effects of acute gastrointestinal haemorrhage, total red-cell volume was measured by using ^{51}Cr-labelled red cells, and all calculations are in terms of red-cell volume, so that no assumption of body-haematocrit/venous-haematocrit ratio is made. In each case the red-cell volume was measured again several months after the acute haemorrhage, when the patient was in good health; this second measurement was assumed to give the patient's normal red-cell volume, and was used in calculating retrospectively the loss of red cells. Additional advantages of the use of the ^{51}Cr technique are that further haemorrhage can be studied quantitatively from the radioactivity of the stools and vomit; confirmation of continuing loss of blood is given by an increase in the rate of disappearance of ^{51}Cr from the circulating blood; and, if blood transfusions are given while there is still measurable radioactivity in the blood, further

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estimates of red-cell volume can be made from the dilution of circulating labelled cells produced by the transfused unlabelled cells.

Patients Studied and Methods of Investigation

Observations were made on patients admitted because of acute gastrointestinal bleeding to the medical wards of two hospitals. Where possible, every patient admitted with a history of recent acute haematemesis or melaena was studied, and there was no selection by reason of severity of haemorrhage. Patients with chronic haemorrhage were not included in this study, nor were patients in whom there was evidence that anaemia had existed before the recent acute loss of blood. One patient in whom initial measurements had been made was excluded from further study because the mean corpuscular haemoglobin concentration was abnormally low at the time of admission, suggesting that previous bleeding might have occurred, resulting in a reduced red-cell volume before the acute haemorrhage. Subsequent observations in health could not be made in four patients, and they also were excluded.

In 30 patients the total red-cell volume was measured, by using ^{51}Cr-labelled red cells, as soon as possible after admission to hospital, before the start of blood transfusion; a further measurement of red-cell volume was made by the same technique two to seven months later, when the patient had recovered good health. Clinical examination was carried out at the time of the volume measurement, and the observations were recorded before the red-cell volume was known. In particular, observations were made of pulse-rate, blood-pressure, colour of the face, temperature of the extremities, and the presence or absence of sweating. Height and weight were measured as soon as possible after admission, and patients were weighed again at the time of subsequent volume measurement in health; surface area was calculated from the formula of Du Bois and Du Bois (1916). The red-cell volume was expressed in litres per square metre, and the volume on admission was calculated as a percentage of the patient's normal red-cell volume.

In 18 patients red-cell survival was studied from observation of ^{51}Cr activity in the circulating blood during five to 27 days after injection of labelled cells. Blood samples were taken at intervals of one to four days; the haematocrit level and radioactivity of each sample were measured. Further measurement of the total red-cell volume was made at the end of the period of study by the injection of red cells labelled with ^{32}P or ^{86}Rb, and in most cases one or two intermediate measurements of red-cell volume were made at the times of transfusion of unlabelled cells. The observed values of ^{51}Cr activity were corrected for change in plasma volume by use of the haematocrit, and also for change in red-cell volume (Turnbull, Hope, and Verel, 1957). As only two to four observations of red-cell volume were obtained in each case, complete corrected survival curves could not be constructed for each patient, but the corrected survival values at times of red-cell volume measurements were compared with normal survival at these times. The intermediate measurements of radioactivity were not used directly

for the estimation of red-cell survival, but they added confirmation, by the smoothness of the curve, to the values observed at the times of red-cell volume estimation. As a normal standard for comparison, ^{51}Cr survival was measured in 10 subjects who were not bleeding and had no haematological abnormality; four to 10 blood samples were taken at intervals of one to seven days after the injection of ^{51}Cr-labelled red cells.

In 18 patients all stools were collected from the time of admission to hospital until no significant radioactivity was present in at least two consecutive stools. Stools were weighed and homogenized with a measured volume of water, and the radioactivity was determined of three weighed portions from each stool homogenate; the total radioactivity in each stool collection was then calculated. A similar procedure was carried out with all vomited material. By comparison with the radioactivity of the circulating red cells, the equivalent volume of red cells in the stools or vomit could be deduced. As the radioactivity of the blood was varying, this calculation involved the assumption of the day on which the haemorrhage occurred. In some cases this assumption could be made with reasonable confidence, but often it was impossible to know when bleeding had occurred, or there might be continuous bleeding during several days. In such cases the calculation was based on the average blood activity over a period of three days before the stool was passed.

The mean rate of red-cell production during the period of observation was calculated from the change in red-cell volume, allowance being made for the total volume of red cells lost in stools, vomit, and blood samples, and for the volume of red cells transfused. The assumption was made that, apart from loss into the alimentary tract, the patient's own red cells, forming the initial red-cell volume, survived normally, so that after t days the initial volume, V_0, would have become $\{V_0.(110-t)/110\}$. The survival after transfusion of cells stored in acid-citrate-dextrose mixture at 4° C depends on the duration of storage (Mollison, 1956). When the duration of storage was less than seven days the survival of transfused cells was considered to be normal, and in calculating the volume remaining after an interval the same factor was applied as with the patient's own cells. When the duration of storage was seven to 14 days it was assumed that the survival at 24 hours after transfusion was 92 per cent., and when the blood had been stored for 15 to 21 days the survival of the red cells at 24 hours was taken as 85 per cent.—an estimate from the graph constructed by Mollison (1956). In all cases the survival of transfused cells after 24 hours was assumed to be normal during the period of study. The use of these survival figures is clearly an approximation, but the error introduced in the calculation of average rate of production of red cells is small.

Example of calculation of rate of production of red cells

Period of study = 22 days.

Initial red-cell volume = 850 ml.

Volume remaining after 22 days $= 850 \times \frac{88}{110}$ = 680 ml. (1)

Volume of red cells transfused (14 days storage)	= 410 ml.	
Survival of transfused cells at 24 hrs. (92 per cent.)	= 377 ml.	
Survival of transfused cells after 22 days $= 377 \times \frac{89}{110}$	= 305 ml.	(2)
Loss of red cells in stools and vomit	= 0	(3)
Loss of red cells in blood samples	= 45 ml.	(4)
∴ Total resulting volume of red cells = (1)+(2)−(3)−(4)	= 940 ml.	
Actual volume of red cells (measured) after 22 days	= 1,700 ml.	
∴ Total volume of new red cells = 1,700−940	= 760 ml.	
∴ Average daily output of red cells $= \frac{760}{22}$	= 35 ml.	

Measurement of total red-cell volume by dilution of labelled red cells. 1. *^{51}Chromium* (Mollison and Veall, 1955). A solution of sodium chromate in isotonic saline, containing 40 to 80 μc ^{51}Cr in 0·05 to 0·2 ml., was added to a glass bottle of capacity 30 ml. fitted with a metal screw-cap in which was a rubber diaphragm. The bottle was then autoclaved. Just before use, a sterile solution of 250 units of heparin or dextran sulphate was added to the bottle. Ten to 20 ml. of blood were withdrawn into a dry syringe and transferred to the bottle. The amount of chromium metal present varied from 0·5 to 3·0 μg per ml. of red cells. The mixture of blood and chromate solution was allowed to stand for 30 to 60 minutes at room temperature, and then the cells were washed twice with cold sterile isotonic saline and resuspended in saline. Twenty ml. of this saline suspension of labelled cells were injected intravenously from a calibrated syringe. Part of the remainder of the suspension of labelled cells was used for the preparation, in duplicate, of a standard dilution in distilled water; part was used for haematocrit measurement in duplicate; and part was centrifuged, and a dilution was prepared of the supernatant fluid. From the radioactivity of the latter solution the total radioactivity injected in the supernatant fluid was calculated. This value was subtracted from the total injected radioactivity in the calculation of total red-cell volume. The radioactivity of the supernatant fluid was less than 2 per cent. of total injected radioactivity. Blood samples, each of about 15 ml., were taken from a vein remote from the site of injection after 15 and 30 minutes. The total red-cell volume was calculated from the dilution of injected labelled cells.

2. *^{32}Phosphorus.* The method of Reeve and Veall (1949) was followed. Allowance was made for the loss of ^{32}P from the red cells by taking three blood samples at intervals of 15 minutes after the injection of labelled cells; the radioactivity was plotted, and the graph extrapolated to give the theoretical activity at the time of injection.

3. *^{86}Rubidium* (Tudhope and Wilson, 1956). The technique was similar to that used with ^{32}P. $^{86}Rb_2CO_3$ in solution in isotonic saline was used, 50 μc being added to 20 ml. of blood and incubated at 37° C for two hours.

4. *Measurement of red-cell volume by blood transfusion.* Compatible whole blood or packed red cells were obtained, and a sample was withdrawn from the bottle for estimation of specific gravity by the $CuSO_4$ method (Phillips, Van Slyke, Hamilton, Dole, Emerson, and Archibald, 1950), and of the haematocrit level. The bottle and contents were then weighed. An intravenous infusion was started with isotonic saline and, when it was apparent that the infusion was running smoothly, the bottle of blood or packed red cells was substituted for the saline bottle. At intervals during the infusion the bottle was gently agitated, to prevent the sedimentation of cells causing the haematocrit value of the infused blood to differ from that of the initial sample taken from the bottle. At the end

of the infusion a small amount (about 50 ml.) of isotonic saline was allowed to run through the tubing, to wash into the circulation all remaining red cells. The bottle and residue were then weighed again, and from the weight, haematocrit value, and specific gravity of the infused blood the total volume of red cells added to the circulation was calculated.

Blood samples were obtained at the beginning and end of the infusion, and the total circulating red-cell volume at the start of the infusion was calculated from the dilution of the circulating labelled cells.

Example: A_1 = radioactivity of circulating blood at start of infusion.
H_1 = haematocrit level „ „ „ „ „
A_2 = radioactivity of circulating blood at end of infusion.
H_2 = haematocrit level „ „ „ „ „
T = volume of red cells infused.
V_0 = total circulating red-cell volume at start of infusion.

$$\frac{A_1}{H_1} \times \frac{H_2}{A_2} = \frac{V_0+T}{V_0}$$

$$V_0 = \frac{T.A_2.H_1}{(A_1H_2-A_2H_1).}$$

The validity of this method was studied in eight subjects with chronic anaemia, in whom it was reasonable to assume that the red-cell volume remained unchanged during a period of a few days. At an interval of one to four days after an initial measurement by the injection of ^{51}Cr-labelled red cells, a further measurement of red-cell volume was made by the transfusion of a measured volume of unlabelled red cells. The discrepancy between the two results is in every case less than 5 per cent. of the red-cell volume (Table I).

TABLE I

Correlation between Measurements of Red-Cell Volume by the Injection of ^{51}Cr-labelled Red Cells and by the Subsequent Transfusion of Unlabelled Red Cells

Case number	*Red-cell volume by injection of labelled cells (litres)*	*Interval (days)*	*Red-cell volume by subsequent transfusion of unlabelled cells (litres)*	*Difference (%)*
1	0·94	4	0·90	−4·3
2	0·71	1	0·71	0
3	0·90	3	0·89	−1·1
4	0·50	1	0·49	−2·0
5	0·42	1	0·41	−2·4
6	0·50	4	0·48	−4·0
7	1·00	1	0·98	−2·0
8	0·35	2	0·35	0

The haematocrit levels of the injected red-cell suspension and of all blood samples were measured in duplicate in Wintrobe tubes centrifuged at 3,000 r.p.m., at a radius of 15 cm., for 55 minutes. Corrections were made for trapped plasma by using the graph of Chaplin and Mollison (1952). In no case was the variation between duplicate haematocrit measurements from the same blood sample, or between haematocrit measurements of duplicate blood samples, greater than 1 per cent.

Measurement of haemoglobin was made with the M.R.C. grey-wedge haemoglobinometer (100 per cent. = 14·8 g. per 100 ml.).

Measurement of radioactivity. Radioactivity due to ^{51}Cr was measured with a scintillation counter (Ekco type N 550). All blood samples were haemolysed with saponin, and 10 ml. were pipetted into a polythene cup, fitting over the

holder of the thallium-activated sodium iodide crystal. Samples of faecal homogenates were poured into weighed polythene cups, which were then weighed again and the weight of faeces determined. Six thousand counts were obtained from all samples (coefficient of variation 1·3 per cent.), except those of low activity, which were counted for 20 minutes. As the technique of homogenization of stools necessitated diluting the faecal radioactivity considerably, the method became inaccurate when the red-cell content of the stools was low. When a faecal homogenate contained about 5 ml. of red cells, only 500 to 600 counts were obtained (coefficient of variation about 5 per cent.). All counts were corrected for radioactive decay. Correction was made for variation in the characteristics of the counter by reference to a standard dilution of ^{51}Cr-labelled cells, which was counted at intervals of every third or fourth sample.

Measurements of ^{32}P and ^{86}Rb were made in a G.M. liquid counter (Veall, 1948). These isotopic labels were used in carrying out final measurements of red-cell volume at the end of the period of study. As there was still ^{51}Cr activity in the red cells at the time, correction was made for the gamma activity from ^{51}Cr recorded by the G.M. tube; this was found to amount to 0·7 per cent. of the counts recorded by the scintillation counter.

Results

Total red-cell volume after haemorrhage. Haemoglobin levels, venous haematocrit levels, and total red-cell volumes at the time of admission to hospital, and several months later after recovery, are shown in Table II. The initial red-cell volume, expressed as a percentage of the volume observed later in health, is plotted in Fig. 1 against the haemoglobin and in Fig. 2 against the haematocrit level at the time of admission. There is not any closer correlation of red-cell volume with the haematocrit level than with the haemoglobin in this series. In chronic anaemia, including cases of chronic haemorrhage, Gibson, Harris, and Swigert (1939) found that the haematocrit level was a better criterion of the degree of deficit of circulating red-cell volume than haemoglobin. In more than half of the cases there had been a severe loss of blood, and in general, when the haemoglobin was less than 11·8 g. per 100 ml. (80 per cent.) or the haematocrit value less than 30 per cent. at the time of admission, the red-cell volume was reduced to less than 50 per cent. of the normal value. The reduction found in haemoglobin and haematocrit values after acute haemorrhage depends partly on the time-interval since haemorrhage occurred, as these values are related to the compensatory increase in plasma volume. In Figs. 1 and 2 the observations are separated into those obtained from patients studied less than 24 hours after the start of haemorrhage and those studied after a longer interval. In 10 of 13 patients studied within 24 hours the haemoglobin was at least 11·1 g. per 100 ml. (75 per cent.), regardless of the depletion of red-cell volume, but in three cases of this group, in which studies were made less than 12 hours after the onset of haemorrhage, the haemoglobin was 8·9 to 9·6 g. per 100 ml. (60 to 65 per cent.). It appears that the rate of haemodilution after acute haemorrhage is variable; the duration of haemorrhage, however, is sometimes difficult to estimate, and in some cases there may have been symptomless haemorrhage into the alimentary tract for several hours before there was evidence that bleeding had occurred.

All the patients studied suffered from acute haemorrhage, and had been apparently in good health previously. In assessing the extent of red-cell loss it has been assumed that immediately before the haemorrhage the haemoglobin and total red-cell volume were normal, and not significantly different from the

TABLE II

Haemoglobin, Venous Haematocrit Level, and Total Red-Cell Volume at Time of Admission to Hospital and Several Months Later after Recovery

					On admission			After recovery			
Case number	*Age (years)*	*Sex*	*Initial weight (kg.)*	*Surface area (sq. m.)*	*Haemoglobin (g./100 ml.)*	*Haematocrit (%)*	*Red-cell vol. (l./sq. m.)*	*Haemoglobin (g./100 ml.)*	*Haematocrit (%)*	*Red-cell vol. (l./sq. m.)*	*Initial red-cell vol. as % of normal*
1	43	M	62·6	1·80	7·1	18·5	0·30	14·4	44·8	1·14	26
2	41	M	62·3	1·78	5·9	19·6	0·38	15·1	39·5	1·17	32
3	42	M	51·2	1·59	8·3	21·6	0·45	14·8	47·0	1·30	35
4	34	M	64·0	1·82	7·6	18·7	0·39	13·8	37·2	1·07	36
5	37	M	73·5	1·87	7·7	22·6	0·37	16·0	43·1	1·01	37
6	30	M	59·4	1·71	6·7	19·6	0·50	16·3	47·8	1·34	37
7	42	F	48·0	1·56	8·6	19·6	0·32	14·2	37·6	0·85	38
8	37	M	69·8	1·84	6·5	16·9	0·43	14·1	41·6	1·10	39
9	44	M	64·3	1·78	12·3	36·2	0·38	14·2	37·4	0·97	39
10	52	M	63·6	1·76	9·2	28·4	0·41	15·2	47·3	1·04	39
11	57	M	90·3	2·11	8·8	27·5	0·43	16·3	43·9	1·08	40
12	25	M	76·8	1·85	6·4	17·2	0·44	16·7	43·8	1·07	41
13	65	M	67·7	1·81	9·9	31·3	0·50	15·8	47·2	1·18	42
14	50	M	68·2	1·91	9·6	26·0	0·46	14·8	42·0	1·07	43
15	44	F	52·3	1·55	6·4	17·3	0·41	13·6	36·8	0·94	44
16	59	F	74·1	1·83	8·0	23·5	0·48	13·9	37·7	1·01	48
17	36	F	51·8	1·50	7·1	21·6	0·47	13·5	41·6	0·98	48
18	45	M	81·5	2·00	11·1	27·2	0·53	15·8	45·8	1·10	48
19	66	M	69·5	1·82	12·1	36·9	0·61	15·8	47·2	1·18	52
20	56	M	70·0	1·88	10·5	27·4	0·63	15·4	43·2	1·21	52
21	36	F	53·5	1·57	12·5	33·0	0·45	15·4	42·4	0·76	59
22	51	M	70·5	1·85	12·3	34·3	0·56	15·4	39·1	0·91	62
23	46	M	61·0	1·71	12·4	31·3	0·70	15·7	42·5	1·07	65
24	58	M	71·3	1·93	12·7	32·8	0·71	15·1	41·0	1·06	67
25	50	M	51·4	1·63	12·6	31·8	0·61	13·6	38·7	0·89	68
26	63	M	70·0	1·85	13·6	35·2	0·82	16·3	42·5	1·13	73
27	66	M	54·0	1·62	12·9	38·6	0·88	14·2	41·5	1·11	79
28	50	F	57·0	1·53	15·1	45·0	0·76	14·8	41·5	0·78	97
29	76	M	57·5	1·70	14·4	41·8	0·85	16·3	39·2	0·85	100
30	64	M	76·2	1·91	15·4	45·3	1·13	16·0	42·2	1·09	104

values observed in health several months later. Support for the validity of this assumption is provided by observations of mean corpuscular haemoglobin concentration. If the red-cell volume had been reduced as a result of previous bleeding, it is probable that the mean corpuscular haemoglobin concentration would have been reduced. Only one patient, whose initial red-cell volume was 39 per cent. of normal, had a low mean corpuscular haemoglobin concentration (22·7 per cent.); this patient was not included in the study.

In Fig. 3 the red-cell volume is plotted against the systolic blood-pressure, and in Fig. 4 against the pulse-rate. Where the systolic blood-pressure was 100 mm. Hg or less there was always a profound reduction in total red-cell volume. There were several cases, however, in which an equally severe or more severe depletion of red cells was associated with a systolic blood-pressure of

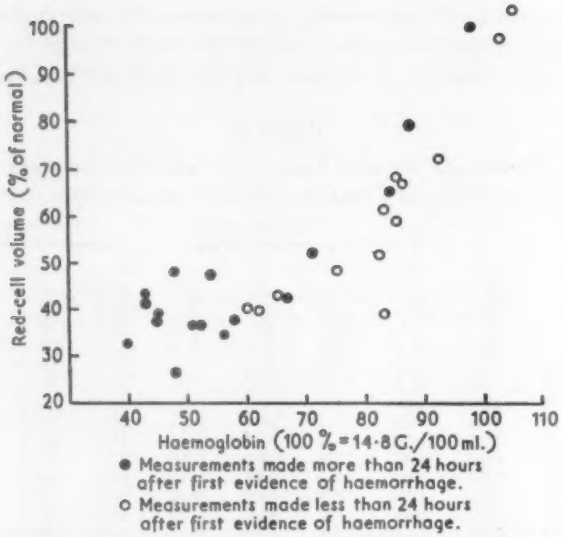

FIG. 1. The red-cell volume on admission, expressed as a percentage of the value measured subsequently in health, is plotted against the haemoglobin on admission.

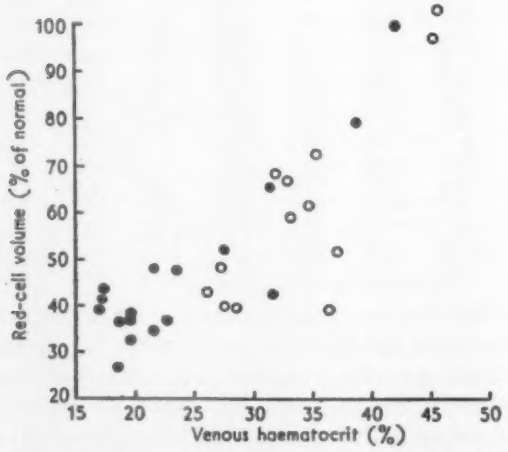

FIG. 2. The red-cell volume on admission, expressed as a percentage of the value measured subsequently in health, is plotted against the venous haematocrit level on admission. Notation as in Fig. 1.

110 to 130 mm. Hg. One patient, who had essential hypertension (usual blood-pressure 200–220/120–130), showed no reduction in systolic pressure, but a lowering of diastolic pressure (200/90), when about half of the total red-cell

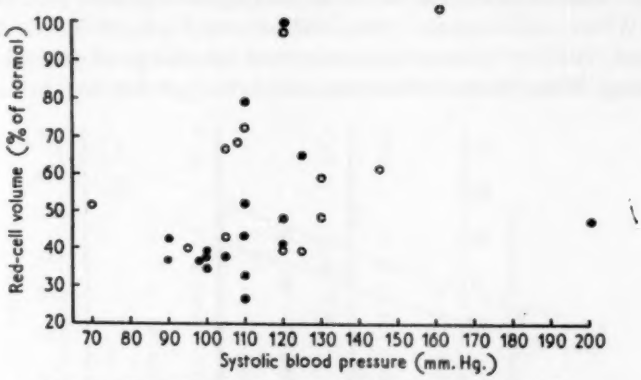

FIG. 3. The red-cell volume plotted against the systolic blood-pressure. Notation as in Fig. 1.

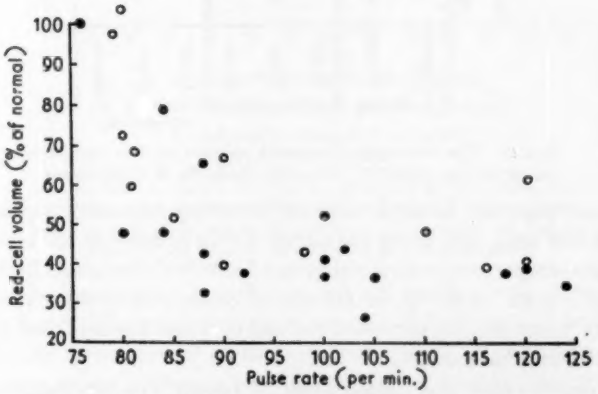

FIG. 4. The red-cell volume plotted against the pulse-rate. Notation as in Fig. 1.

volume had been lost. The pulse-rate bore some relationship to red-cell volume, in that most patients whose pulse-rate was greater than 110 per minute had suffered severe blood loss; but, on the other hand, a pulse-rate of 80 to 90 did not preclude a very low red-cell volume. The relationship between red-cell volume and the presence of sweating, pallor of the face, and coldness of the extremities, is shown in Fig. 5. These signs are not by themselves of great value in assessing the extent of blood loss, except that the severest degrees of pallor and coldness are associated with a red-cell volume reduced by 50 per cent. or more. The absence of coldness of the extremities does not exclude the possibility of severe loss of blood.

Further haemorrhage and red-cell survival. Red-cell survival and the occurrence

of further haemorrhage were studied in 18 patients. Measurements of ^{51}Cr survival, corrected for change in haematocrit level and in total red-cell volume, are shown in Fig. 6, in which is also shown the range of ^{51}Cr survival in 10 normal subjects. These normal results are in good agreement with published figures (Read, Wilson, and Gardner, 1954; Mollison and Veall, 1955; Turnbull, Hope, and Verel, 1957). ^{51}Cr survival uncorrected for change in red-cell volume is misleading. When the red-cell volume is becoming greater, as it is in most cases

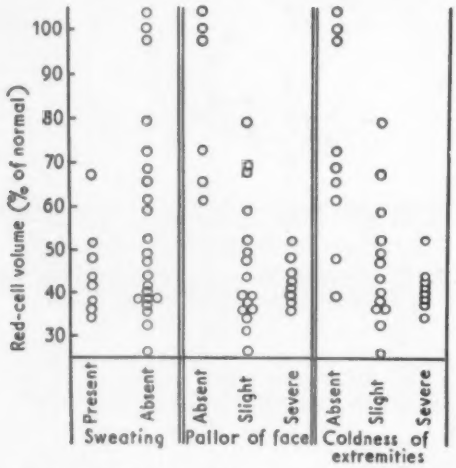

FIG. 5. The relation of red-cell volume to the presence of sweating, pallor of face, and coldness of extremities.

after haemorrhage, the labelled cells are becoming increasingly diluted by the addition of new cells, and hence the curve of ^{51}Cr activity in the circulating red cells becomes steeper, suggesting a shortened survival (Strumia, Taylor, Sample, Colwell, and Dugan, 1955). In the absence of haemolysis or other haematological abnormality, shortening of corrected red-cell survival was regarded as an indication of continuing haemorrhage.

Further haemorrhage and radioactivity of stools. The occurrence of further haemorrhage was studied also by direct measurement of the total radioactivity contained in the stools. In eight patients there was no significant radioactivity in the stools during 14 days, so that continued bleeding, if any, must have been very slight; the red-cell survival was normal (Fig. 6). In four patients slight loss of blood continued after the injection of labelled cells, but the total radioactivity in the stools during 14 days was equivalent to less than 20 ml. of red cells in each case; thus the average daily loss was small, and the red-cell survival was within normal limits (Fig. 6). The 'normal' ^{51}Cr survival curve does not, of course, represent completely normal conditions, as the blood samples necessary to define the curve constitute minor recurring haemorrhages. If five blood samples, each of 15 ml., are withdrawn from a normal subject during a period of 30 days, the total red-cell loss is about 30 ml., an average loss of about one ml. of red cells a day. The additional loss of red cells in the stools must be considerably greater

than this basic loss before the ^{51}Cr survival is shorter than the normal range. One patient lost a total of 86 ml. of red cells in the stools in 23 days; the ^{51}Cr survival in this case was 47 per cent. at 23 days, which is at the lower border of the normal range. These last five patients appeared to have minor bleeding persisting over a period of many days after the acute haemorrhage.

Five patients suffered a second major haemorrhage after admission to hospital, the radioactivity of the stools and vomited material corresponding to a loss of

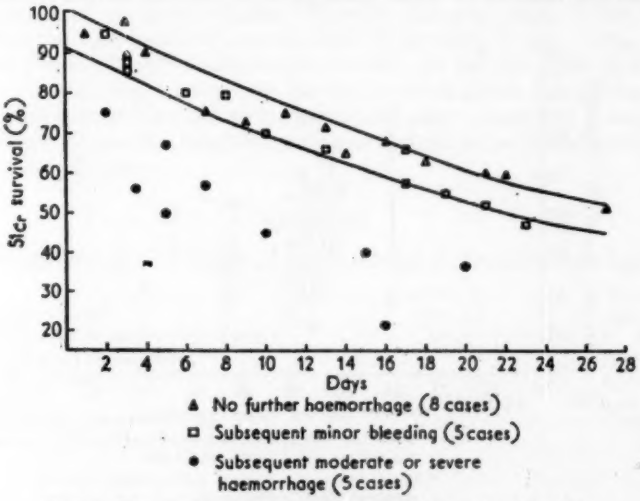

FIG. 6. The survival of ^{51}Cr in circulating red cells. Observations are corrected for the change in haematocrit level and in total red-cell volume. The range of values observed in 10 normal subjects is also indicated.

200 to 800 ml. of red cells. In each case red-cell survival was abnormally short (Fig. 6). In three of these patients there was clinical evidence of further haemorrhage; after an initial period of improvement following their admission there was increasing pallor, rising pulse-rate, and falling blood-pressure, and in one case haematemesis. In two patients, in whom the calculated losses of red cells in the stools were 280 ml. and 300 ml. respectively, none of these clinical signs appeared, although it was noted that the haemoglobin level did not rise as quickly as was expected during two weeks after the initial haemorrhage.

Red-cell production following haemorrhage. Calculation of the average daily rate of red-cell production during five to 23 days after the acute haemorrhage was made in 15 patients (Fig. 7). If the normal red-cell life is assumed to be 110 days, the average rate of production of red cells in normal subjects is 14 to 20 ml. per day. There is a relationship between increase in the rate of production and diminution of the red-cell volume, which is most apparent in patients who had no transfusions. Patients who received transfusions had a considerably lower rate of production than those with a similar initial red-cell volume who were treated without transfusion. If, however, the red-cell production in cases of

transfusion is plotted against the red-cell volume after transfusion, there is an inverse relationship between red-cell volume and red-cell production, which applies both to patients who had transfusions and to those who did not (Fig. 7). The production rates observed after the severest haemorrhages were about three to four times the normal. Two cases of gastrointestinal haemorrhage provide an interesting comparison (Table III). The initial red-cell volumes were very similar, and in both cases the red-cell volume had increased by approximately 100 per cent. in 22 days. Both patients received ferrous gluconate, 2 g.

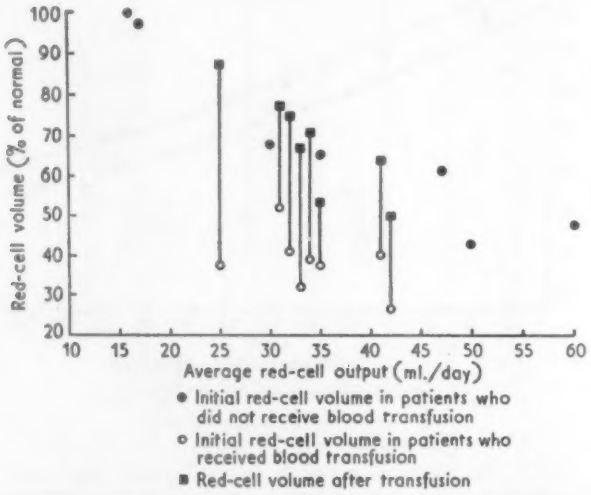

FIG. 7. The relation of average red-cell output to red-cell volume.

daily, during the period of study. One patient received a blood transfusion of 420 ml. of red cells, and the other had no transfusion. The rate of red-cell production had clearly been much less in the former patient.

Reticulocytes in the peripheral blood were counted in 10 cases on alternate days for 14 days after the acute haemorrhage (Table IV). In general, the increase in reticulocytes reflects the increased rate of production of red cells and the depletion of red-cell volume, but the reticulocyte percentage is not a reliable guide to the extent of the erythropoietic response. In interpreting the reticulocyte percentage in severe loss of blood allowance must be made for the effect of reduced red-cell volume.

Chronic respiratory disease and acute alimentary haemorrhage. In four cases in this series there was chronic respiratory disease: pneumoconiosis and emphysema in Cases 13 and 19, and chronic bronchitis and emphysema in Cases 3 and 11. The clinical signs after acute haemorrhage were of the severest degree in all these cases, although the red-cell volume depletion was not as extreme as in some others. In each case there was profuse sweating, severe pallor and coldness of the extremities, and reduced systolic blood-pressure (70, 90, 95, and 100 mm. Hg),

and in two cases tachycardia of 120 per minute. One further case, not otherwise included in the present study, is of considerable interest.

A bricklayer, aged 47 years, was admitted after an apparently severe haematemesis. He complained of severe dizziness and faintness, his hands and feet were intensely cold, and there was profuse sweating and conspicuous cyanosis of the face and extremities. The blood-pressure was 90/75, and pulse-rate 130. The haemoglobin was 23·7 g. per 100 ml. (160 per cent.), and the haematocrit level 70 per cent. The red-cell volume at the time of admission was 4·2 litres; subsequent estimations, on three occasions after his recovery, were 5·3, 4·9, and 5·3 litres. Thus the initial red-cell volume was 82 per cent. of this patient's usual volume, and was still very much above normal; yet he had signs which, apart from the cyanosis, might have been associated with severe loss of blood. The results of respiratory function tests, performed later, suggested a very severe degree of emphysema; the polycythaemia was thought to be secondary to chronic respiratory insufficiency.

TABLE III

Comparison of Two Cases of Acute Gastrointestinal Haemorrhage

	Case A	*Case B*
Initial red-cell volume	880 ml.	850 ml.
Volume of red cells in stools and vomit	86 ml.	0
Volume of red cells transfused	0	420 ml.
Final red-cell volume	1,670 ml.	1,700 ml.
^{51}Cr survival	47% in 23 days	60% in 22 days
Maximum reticulocyte percentage	8%	4%
Average rate of red-cell production (ml./day)	50	35

TABLE IV

Maximum Reticulocyte Percentage, Red-Cell Volume, and Average Rate of Red-Cell Output

In patients who received transfusions the red-cell volume after transfusion is given, with the pre-transfusion volume in parenthesis.

Case number (*Table II*)	*Red-cell volume* (% *of normal*)	*Maximum reticulocyte level* (%)	*Average rate of output of red cells* (*ml./day*)
29	100	1	16
28	97	1	17
7	87 (38)	2	25
12	75 (41)	6	32
25	68	4	30
23	65	4	35
11	64 (40)	6	41
22	62	5	47
6	53 (37)	6	35
14	43	8	50

Discussion

In many studies of blood loss after haemorrhage the total blood volume and red-cell volume have been derived from the venous haematocrit values, and the plasma volume measured by Evans-blue dye. In normal subjects, with this method, red-cell volume is overestimated by about 15 per cent., owing to the difference between body haematocrit and venous haematocrit values (Gibson,

Peacock, Seligman, and Sack, 1946; Reeve, 1948; Barnes, Loutit, and Reeve, 1948). Calculation of total blood volume from either plasma or red-cell volume requires knowledge of the true proportion of total red cells and total plasma in the circulating blood—the body haematocrit level. This can be derived from the venous haematocrit level if the body-haematocrit/venous-haematocrit ratio is known, as it is in normal subjects (Reeve and Veall, 1949; Wasserman, Yoh, and Rashkoff, 1951; Verel, 1954; Hicks, Hope, Turnbull, and Verel, 1956), but it cannot be assumed that this ratio will be normal after acute haemorrhage, especially where there is circulatory upset with hypotension and peripheral vasoconstriction. After acute gastrointestinal haemorrhage Hope and Verel (1955) found a low ratio in six cases, and Tibbs (1956) found the ratio to be abnormal and variable. Gibson, Seligman, Peacock, Fine, Aub, and Evans (1947) showed that, in dogs, the dye-haematocrit method gave a greater overestimate of red-cell volume in the presence of shock than in the normal state; Grant and Reeve (1951) deduced that, if these results can be applied to man, after severe haemorrhage the overestimate of red-cell volume by the dye-haematocrit method is of the order +35 per cent. Noble and Gregersen (1946) calculated red-cell volume from plasma volume and the arterial haematocrit level, but Hope and Verel (1955) have shown that the body-haematocrit/arterial-haematocrit ratio is not constant, and that there is no advantage in using the arterial haematocrit in measurement of blood volume.

In the present study red-cell volume has been measured directly by using red cells labelled with ^{51}Cr, and all calculations are in terms of volume of red cells. This method avoids any assumption regarding body haematocrit levels. Moreover, the injection of the patient's own red cells labelled with ^{51}Cr enables observations to be made of further haemorrhage from measurement of radioactivity of the stools; also red-cell survival can be studied, giving further information about persisting or recurring haemorrhage; and further measurement of red-cell volume can be made during several weeks after the initial injection, by transfusion of a measured volume of unlabelled cells into the pool of circulating labelled cells.

Several groups of workers studied blood loss resulting from injury during the 1939–1945 war, and found that in many cases the depletion of blood volume was considerably greater than was expected, and that in severe injuries over 50 per cent. of the total blood volume might be lost (Emerson and Ebert, 1945; Noble and Gregersen, 1946; Grant and Reeve, 1951). Similar extensive loss of blood has been found in severe trauma in civilians (Evans, Hoover, James, and Alm, 1944; Clarke, Topley, and Flear, 1955). In 1938 Bennett, Dow, Lander, and Wright reported that in severe cases of gastroduodenal haemorrhage red-cell volume was reduced by over 50 per cent. They calculated red-cell volume from venous haematocrit levels and plasma volume as measured by Congo red. Tibbs (1956), using mainly the Evans-blue technique in the study of gastroduodenal haemorrhage, estimated that in many cases 2,000 to 4,000 ml. of whole blood had been lost. In the present study 17 of 30 patients were found to have lost over 50 per cent. of their total red-cell volume.

The level of circulating haemoglobin is not of great value in assessing the extent of haemorrhage, except that in a previously healthy subject, with normal peripheral blood, a reduction of haemoglobin to less than 11·8 g. per 100 ml. (80 per cent.) indicates severe loss of blood. The haemoglobin level and the venous haematocrit are not reliable guides to blood loss, because haemodilution may occur rapidly, but the rate of dilution is variable and unpredictable (Dyson, Plaut, and Vaughan, 1944; Grant and Reeve, 1951; Ellison, Zollinger, Cedars, and Britt, 1953). This fact is exemplified in the present results, since in some patients, with severe depletion of red cells, the haemoglobin had fallen to 8·9 to 9·6 g. per 100 ml. (60 to 65 per cent.) in less than 12 hours, whereas in one patient, with an equally severe red-cell loss, the haemoglobin was 12·7 g. per 100 ml. (86 per cent.) after 48 hours.

Most workers have agreed that in traumatic blood loss the clinical sign which correlates best with the reduction of blood volume is the systolic blood-pressure (Keith, 1919; Robertson and Bock, 1919; McMichael, 1941; Kekwick, Maycock, Marriott, and Whitby, 1941; Emerson and Ebert, 1945; Grant and Reeve, 1951). The blood-pressure is related less to the volume of blood lost than to the actual blood volume, which depends on the extent to which the plasma volume has been increased by haemodilution. Grant and Reeve (1951), studying traumatic haemorrhage, found that when the blood loss was more than 40 per cent. the systolic blood-pressure was usually 100 mm. Hg or less. In the present investigation a systolic blood-pressure of 100 mm. Hg or less was always associated with loss of at least 50 per cent. of total red-cell volume. It is also apparent, however, that a systolic blood-pressure of above 100 mm. Hg does not necessarily indicate a smaller blood loss. Tibbs (1956) formed similar general conclusions about the value of the systolic blood-pressure in assessing the severity of gastroduodenal haemorrhage. He found that the diastolic blood-pressure showed a closer relationship to blood-volume depletion than the systolic pressure. This was not found to be the case in the present study. The pulse-rate does not bear a close relationship to the blood volume after haemorrhage, although there is a general trend to increase in the pulse-rate with reduction of blood volume (Keith, 1919; Emerson and Ebert, 1945; Grant and Reeve, 1951; Tibbs, 1956), which is shown in the present results.

The quantitative study of alimentary haemorrhage by measuring ^{51}Cr activity in faeces and blood depends on the assumptions, first, that after the intravenous injection of the patient's own red cells labelled with ^{51}Cr an insignificant amount of ^{51}Cr passes into the alimentary tract, apart from haemorrhage; and secondly, that if labelled red cells enter the bowel, the ^{51}Cr is not reabsorbed, but is excreted quantitatively in the stools. Evidence in support of these assumptions has been obtained from studies in dogs (Owen, Bollman, and Grindlay, 1954), rabbits (Donohue, Motulsky, Giblett, Pirzio-Biroli, Viranuvatti, and Finch, 1955), and man (Roche, Perez-Gimenez, Layrisse, and Di Prisco, 1957).

In the present study the maximum reticulocyte response observed was 8 per cent. Schiødt (1938) reported that in patients who were not anaemic before gastrointestinal haemorrhage the ensuing reticulocytosis rarely exceeded 10 per

cent. The maximum increase observed in the rate of production of red cells after acute haemorrhage in the present series was three to four times the normal. This is the same level of response as occurs during early remission from pernicious anaemia under the action of specific therapy (Finch, Coleman, Motulsky, Donohue, and Reiff, 1956). It is presumably not a maximal erythropoietic response, since in chronic haemolytic anaemia Crosby and Akeroyd (1952) have calculated that the rate of red-cell production may be increased seven to eight times the normal; a similar rate of production was found, in unpublished studies with ^{51}Cr in this laboratory, in cases of congenital spherocytic anaemia and acquired haemolytic anaemia. In the present study, however, average rates of production over a period of five to 23 days have been calculated, and the red-cell output is probably greater during part of this period. There is an inverse relationship between the rate of red-cell production and the red-cell volume. The increase in red-cell volume produced by blood transfusion results in diminution or inhibition of the erythropoietic response to haemorrhage. Birkhill, Maloney, and Levenson (1951) gave blood transfusions to normal subjects, and found diminution of erythropoiesis; this effect increased with the volume of blood transfused, and an almost complete cessation of red-cell production followed a 40 per cent. increase in red-cell volume.

There is no doubt that adequate blood transfusion is of urgent necessity in many cases of acute gastrointestinal haemorrhage in order to restore the greatly depleted red-cell and total blood volumes. In planning blood transfusion in such cases, the possibility of severe depletion of red-cell volume to less than 50 per cent. of normal must be considered, especially if the haemoglobin is less than 11·8 g. per 100 ml. (80 per cent.), the systolic blood-pressure less than 110 mm. Hg, or the pulse-rate more than 110 per minute. Transfusion of 2·3 litres (five pints) of stored blood is necessary to increase the red-cell volume by about one litre, assuming that the immediate survival of the transfused cells is complete. The situation is different, however, during the convalescent stage, when the red-cell volume is no longer grossly diminished and the total blood volume is probably normal. Blood transfusion is sometimes given at this stage with the aim of speeding recovery; when considering this treatment it should be realized that one result of transfusion may be to diminish the rate of red-cell production, so that after two or three weeks the total increase in red-cell volume will be the same whether or not transfusion is given.

I am very grateful to Professor G. M. Wilson for advice and helpful criticism during the performance of this work. I wish to thank the physicians of the Royal Infirmary, Sheffield, who allowed me to study patients admitted under their care; Dr. E. K. Blackburn and the staff of the Haematology Department, the Royal Infirmary, Sheffield, who performed the reticulocyte counts and some of the haemoglobin estimations; and Dr. K. Hardy, Medical Superintendent, Wharncliffe Hospital, Sheffield, for his co-operation. I gratefully acknowledge a grant towards the purchase of radioisotopes from the Endowment Fund of the United Sheffield Hospitals.

Summary

1. The advantages of ^{51}Cr as a red-cell label in the study of acute haemorrhage are discussed.

2. Studies of red-cell volume, using ^{51}Cr-labelled red cells, are reported in 30 patients with acute gastrointestinal haemorrhage. In 18 patients the red-cell volume was reduced by more than 50 per cent.

3. The correlation between depletion of red-cell volume and clinical findings is studied. In previously healthy subjects haemoglobin levels of less than 11·8 g. per 100 ml. (80 per cent.) at the time of admission are usually associated with loss of more than 50 per cent. of red-cell volume. A systolic blood-pressure of less than 110 mm. Hg, and a pulse-rate of more than 110 per minute, are usually associated with a similar degree of blood loss, but there are exceptions. Several patients who had severe loss of blood did not show abnormalities of systolic blood-pressure or pulse-rate at the time of admission.

4. Further haemorrhage was investigated by direct measurement of radioactivity in the stools, and by study of the rate of disappearance of ^{51}Cr from the circulating blood. Observations of ^{51}Cr survival were corrected for variation in red-cell volume, by using subsequent measurements of red-cell volume by ^{32}P- or ^{86}Rb-labelled red cells or the transfusion of unlabelled red cells into a labelled pool.

5. The average rate of red-cell production was calculated in 15 patients from the change in red-cell volume, allowing for the volume of red cells transfused or lost in stools and vomit. The rate of red-cell output is in inverse relation to the red-cell volume. There is evidence that blood transfusion diminishes or inhibits the increased erythropoiesis in response to haemorrhage. The maximum erythropoietic response observed was three to four times the normal.

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THE ASSOCIATION OF PHYSICIANS OF GREAT BRITAIN AND IRELAND

1958

FIFTY-SECOND ANNUAL GENERAL MEETING

THE FIFTY-SECOND ANNUAL GENERAL MEETING was held at the Riley Smith Hall, the Union, Leeds, on Friday and Saturday, May 23 and 24. The attendance book was signed by 199 members and 14 visitors.

The President, Professor Sir L. S. P. Davidson, was in the Chair.

The Minutes of the last Annual General Meeting, having been published in the *Quarterly Journal of Medicine*, were taken as read, confirmed, and signed. The following Officers, Executive Committee, and Honorary, Senior, and Ordinary Members were elected unanimously, as was the new member of the Editorial Board:

Executive Committee

President: Dr. J. R. H. Towers.

President-Elect: Professor Harold Scarborough.

Treasurer: Dr. C. M. Fletcher.

Secretary: Dr. R. I. S. Bayliss.

Members for England and Wales—
Dr. W. Brockbank.
Dr. J. S. Richardson.
Dr. D. V. Hubble.
Dr. K. Robson.
Dr. C. N. Armstrong.
Professor A. Kekwick.

Members for Scotland—
Dr. I. Gordon.
Professor D. M. Dunlop.
Dr. J. H. Wright.

Members for Ireland—
Dr. Brendan O'Brien.
Dr. J. T. Lewis.
Dr. M. G. Nelson.

Election of Honorary Member

Professor Sir L. S. P. Davidson.

Election of Senior Members

Dr. H. Gainsborough.
Dr. J. H. Sheldon.
Dr. J. A. Smythe.

Election as Member of the Editorial Board

Professor M. L. Rosenheim.

Election of Ordinary Members

Alister James Victor Cameron, M.D., M.R.C.P., Consultant Physician, Western Infirmary, Glasgow.

James Findlay Dow, M.B., F.R.C.P., Assistant Physician, St. George's Hospital.

Rees Byron Evans, M.D., F.R.C.P., Physician, United Cardiff Hospitals.
Ian William Ballantyne Grant, M.B., F.R.C.P., Consultant Physician, Respiratory Diseases Unit, Northern General Hospital, Edinburgh.
John Duncan Hay, M.D., F.R.C.P., Professor of Child Health, University of Liverpool.
James Caldwell Houston, M.D., F.R.C.P., Assistant Physician, Guy's Hospital.
John Stephens Logan, M.D., M.R.C.P., Physician, Royal Victoria Hospital, Belfast.
John Melville Malins, M.B., F.R.C.P., Physician, United Birmingham Hospitals.
John Wylmer Paulley, M.D., M.R.C.P., Consulting Physician, Ipswich Hospital.
Peter Carew Reynell, D.M., M.R.C.P., Consultant Physician to the Royal Infirmary and St. Luke's Hospital, Bradford.
John Marcus Stowers, M.D., M.R.C.P., Senior Lecturer in Medicine, Queen's College, University of St. Andrews.
Ronald Bolton Thompson, M.D., M.R.C.P., Lecturer in Medicine, King's College, University of Durham.

The Treasurer, in presenting his accounts, pointed out that the opening balance for the year had been £1,148. 4*s*. 9*d*. and the closing balance £1,088. 16*s*. 9*d*. He suggested that this was satisfactory if it were remembered that the Association had contributed an extra £308 to the Oxford University Press to provide additional pages for the *Quarterly Journal of Medicine*. He thought that even with the new rate of members' subscriptions for the *Journal* the finances of the Association would be in a better position, as there would also be a permanent increase in the size of the *Journal*, and this should avoid calls for extra payments in future. With this belief in the continued soundness of the financial position, the Executive Committee recommended that there should be no increase in the subscription to the Association.

Place of Meetings, 1959 and 1960. Professor Scarborough's invitation to meet in Cardiff in 1959 was reaffirmed and again accepted. The Meeting in 1960 will be held in London.

Amendment to Rule 2. The Committee suggested that a new category of Overseas Ordinary Members might enable graduates of the Universities of Great Britain and Ireland, who took up posts in Universities throughout the Commonwealth, to enjoy belonging to the Association and to arrange to attend the Association's meetings when on visits to this country. It was suggested that Ordinary Members of the Association who were at present abroad could be transferred to this new category of membership, thereby creating a few extra vacancies for Ordinary Members. After some discussion as to the number of Overseas Ordinary Members who should be elected, the following amendment to the first paragraph of Rule 2 was agreed:

> 'It shall consist of Ordinary Members, Overseas Ordinary Members, Honorary, and Overseas Honorary Members not exceeding five in number. It shall contain not more than 350 Ordinary Members and 20 Overseas Ordinary Members, who shall be Physicians actively engaged in the Teaching of Medicine, or in Research. Men of distinction in medicine, who have contributed to its advancement, are eligible as Honorary Members.'

The newly elected President, Dr. J. R. H. Towers, then took the Chair.

SCIENTIFIC BUSINESS

Friday Morning, May 23

1. Dr. F. F. Hellier (introduced) said that medicine in Leeds really started in 1767, when the Infirmary was founded largely through the enthusiasm of William Hey. It was called the General Infirmary because the founders intended it for patients from everywhere, not only from Leeds. The Infirmary began in an old house, but the first real hospital was built in 1771, and was not superseded until 1867, when the present Infirmary was opened. This was one of the first hospitals to be built on the pavilion system, and its resemblance to St. Pancras Station is due to the common architect—Gilbert Scott. The Medical School, developing out of the rival Anatomy Schools of Thackrah and Sam Smith, was founded in 1831 in a house to which the rowdy medical students were only allowed to enter by the back door. In 1865 Sir James Paget opened the first building erected expressly as a Medical School in this country. The final move to the present site took place in 1894, and in 1904 the Medical School became the Faculty of Medicine in the newly established University of Leeds. William Hey, the father of Leeds surgery, studied under John Hunter; he was a distinguished anatomist and surgeon, an eminent citizen, and a friend of Joseph Priestly.

In 1801 he gave a course of demonstrations to the public, in which he dissected a human body, ladies being admitted to the final lecture on the eyes. It was so successful that he repeated it three times in subsequent years. Thackrah, the main founder of the Medical School, is best known for his book on Occupational Diseases, the first of its kind in this country and the product of tireless investigation. He was a brilliant but difficult man, and died a year after the school was formed. Allbutt was the most distinguished physician of Leeds; he retired in 1884 after his allotted 20 years on the staff. After a short period as a Commissioner in Lunacy, he became Regius Professor of Physics at Cambridge until 1925. Whilst at Leeds he introduced the short clinical thermometer, advanced the use of the ophthalmoscope in general medicine, and was the meticulous secretary of a notable medical club. Within a few weeks of his death at 87 he was able to discuss with zest the newly discovered insulin. He knew the Brontës, and is said to have been the inspiration for George Eliot's Lydgate. Other notable members of the medical club were Teale, an eye surgeon and an F.R.S. for his work in Public Health, Wheelhouse, McGill who removed the first prostate, and Jessop who presented cases of Paget's disease 10 years before Paget described them. It was Moynihan who made Leeds a Mecca for foreign surgeons. DR. HELLIER recalled that, as one of his last dressers, he remembered Moynihan as a superb technician, never perturbed or raising his voice in a crisis, a magnificent orator and a commanding figure. Having shown that Leeds had a great past, DR. HELLIER and his appreciative audience agreed that it also has a great future.

2. DR. S. G. OWEN (introduced) and PROFESSOR G. A. SMART discussed *Thyroid Antibodies in Myxoedema*. Over 400 patients with various thyroid disorders had been investigated with an adaptation of the tannic acid haemagglutination technique. The method depended on the fact that red blood-cells treated with tannic acid would adsorb protein by agglutinating in its presence. With protein extracted from human thyroid gland coating the cells, this test had proved more sensitive and more quantitative than precipitin reactions, and had revealed antibodies to thyroid protein in 80 per cent. of patients with apparently spontaneous myxoedema. The results in 69 cases of undoubted myxoedema without thyroid enlargement were presented: antibody concentrations were usually below the level required for a positive precipitin test, and were significantly less than those found in 27 patients with Hashimoto's thyroiditis of recent origin. They were, however, strikingly similar to the concentrations in 33 further cases of Hashimoto's disease which had been tested more than a year after subtotal thyroidectomy and the institution of treatment with oral thyroid extract. Moreover, the overall proportion of patients with Hashimoto's disease, both recent and long-standing, having antithyroid antibodies in some degree, was identical with that in myxoedema. An adequate follow-up study in individual cases had not yet been possible, but, as no difference had been noted between levels in untreated patients and in those who had received adequate doses of thyroid extract for periods of up to eight years, it seemed unlikely that treatment had much effect on the antibody concentration in myxoedema.

PROFESSOR E. J. WAYNE said that he had investigated the response of normal individuals and of patients suffering from thyroid disease to the intradermal injection of an antigen made from the glands of thyrotoxic patients. Positive skin reactions were found only in patients with Hashimoto's thyroiditis and 'primary' myxoedema, that is patients with no enlargement of the thyroid gland. These findings agreed with those of Dr. Owen, and suggested that auto-immunization may be a factor in some cases of 'primary' myxoedema. He commented that it would be interesting to see whether these positive skin reactors gave positive reactions with the tanned red cell technique.

DR. A. G. MACGREGOR said that, using the tanned red cell technique, he also had found a high incidence of positive results in patients with 'primary' myxoedema.

3. DR. C. L. COPE discussed *The Choice of Adrenal Function Tests*, and said that a greater effort should be made to adapt adrenal function tests to the varied circumstances for which they were now needed. Blood levels suffered from the disadvantages that they were technically difficult and were subject to wide fluctuations during the day. Urinary analyses gave a more average figure, which was an advantage clinically. When a variety of urinary indices were compared, the urinary hydrocortisone level was found to rise in response to ACTH stimulation twice as far as did the next most sensitive index, the level of 17-ketogenic steroids. In a group of 12 cases of Cushing's syndrome the urinary hydrocortisone averaged eight times the normal, compared with only four times for the ketogenic steroids, and the overlap for the former between normal subjects and patients with Cushing's syndrome was much smaller than with 17-ketogenic steroids. In Cushing's syndrome

adrenal inhibition by exogenously administered steroids was also more clearly demonstrated by a decrease in urinary hydrocortisone than by either keto-steroids or ketogenic steroids, which decreased much more slowly and to a lesser degree. Using ^{14}C-labelled hydrocortisone, Dr. Cope had developed a method for measuring the actual rate of hydrocortisone production in the human patient. Two patients were reported who were insensitive to ACTH stimulation, but nevertheless had normal secretions of hydrocortisone. A low urinary hydrocortisone output was not evidence of hypoadrenalism, and this condition was best revealed by a low urinary output of tetrahydro metabolites of hydrocortisone. Urinary hydrocortisone excretion correlated poorly with hydrocortisone production, and urinary 17-ketosteroid output bore no relation to production, but urinary 17-ketogenic steroid excretion correlated best, though only in states of activity above the normal range. In these circumstances about half the hydrocortisone produced was excreted as 17-ketogenic steroids.

Dr. P. Fourman inquired what was the excretion of the metabolic derivatives of cortisol in patients with wasting diseases, and with some psychological disorders, who had abnormally low excretion of 17-ketosteroids and 17-ketogenic steroids.

Dr. Cope said he had studied only a few wasted subjects and no psychiatric patients. Excretion of metabolites might be reduced, but hydrocortisone production was sometimes above normal in such subjects.

Dr. J. D. N. Nabarro thought that estimations of cortisol and its tetrahydro metabolites would rarely be practicable in routine hospital laboratories. He had found measurement of urinary 17-hydroxycorticosteroids by the Norymberski techniques satisfactory for diagnostic purposes. He also advised the administration of ACTH by the intramuscular route rather than intravenously, because of the danger of reactions.

In reply, Dr. Cope pointed out that Dr. G. W. Thorn at Harvard had found the intravenous route more certain in action than the intramuscular, and that Thorn and his colleagues had this year published a series of seven cases of patients with some clinical evidence of Addison's disease who continued to produce steroids in the urine but were insensitive to ACTH stimulation.

4. Dr. Wilfrid Oakley described the *Action of Prednisone in Severe Insulin Resistance*, and said that administration of prednisone might reduce the insulin requirements of diabetic patients whose daily dose was as high as 1,000 units. The existence of an insulin-inhibiting substance, or substances, had been studied by the mouse convulsion technique, passive cutaneous anaphylaxis, and the glucose uptake of the isolated rat hemi-diaphragm. With the passive cutaneous anaphylaxis procedure all but two of 89 controls gave negative results, while positive results were obtained in nine out of 10 cases showing insulin resistance. Treatment with prednisone in two cases of insulin resistance with a positive passive cutaneous anaphylaxis test induced a dramatic fall in insulin requirement, whereas in a third case, in which this test was negative, prednisone significantly increased the insulin requirement. The discrepancy between the results obtained by the passive cutaneous anaphylaxis and the rat-diaphragm test was considered as possible evidence for the presence of more than one insulin antagonist. Further investigation by fractionation of the plasma proteins in cases of insulin resistance was considered as a possible method of demonstrating the simultaneous presence of more than one insulin-inhibitory substance.

Dr. J. D. N. Nabarro gave some more details of one of the patients reported by Dr. Oakley. During the first four days of prednisone treatment his diabetes deteriorated, but a reduction in insulin resistance was obvious by the seventh day. Dr. Nabarro also mentioned a patient in whom insulin resistance decreased while tolbutamide was being given.

Dr. John Naish described a patient with severe rheumatoid arthritis, who developed diabetes mellitus in 1953, and was found in 1954 to need 100 units of insulin daily. By early 1955 insulin requirements had risen to 760 units daily. Oral cortisone acetate, 150 mg. per day, reduced the insulin requirements to 80 units daily but, on discontinuing the steroid after one month, insulin requirements rose again to nearly 1,000 units, this dose being necessary to clear ketosis, though not sufficient to control glycosuria. On recommencing cortisone treatment, insulin requirements again fell to below 100 units daily, and the patient continued this treatment until he died one year later from bronchopneumonia.

5. Drs. G. N. Chandler (introduced) and G. Watkinson described the value of hourly gastric aspirations through an indwelling tube in the *Early Diagnosis of Haematemesis*. Two hundred and thirty-four patients admitted in emergency after haematemesis had been

investigated by this technique. Blood-staining of the aspirated samples often provided advance information of recurrent haemorrhage, and occasionally indicated the site of the bleeding. A study of the pH of the gastric juice showed that characteristic secretion patterns accompanied bleeding in both acute and chronic peptic ulcer. A previously unreported finding was achlorhydria in patients with a bleeding acute peptic ulcer. These secretory abnormalities were sufficiently clear-cut to be of diagnostic value in most patients. Gastroscopy was performed in 36 patients in the acute stages of their illness, and examinations, combined with the information obtained from secretion studies, allowed some tentative conclusions as to the aetiology of gastritis.

Professor L. J. Witts inquired whether Dr. Chandler had had the opportunity of carrying out suction biopsy of the stomach in the cases of acute ulceration with achlorhydria.

Dr. P. Hugh-Jones asked whether the rate of gastric emptying had been measured.

Dr. Charles Fletcher inquired whether the tube had been advanced into the duodenum to distinguish between gastric and duodenal bleeding.

Dr. A. H. James said that, while useful information could be obtained by sampling the gastric contents of patients with haematemesis, a disadvantage was that occasionally the presence of the tube appeared to predispose to a lower respiratory infection. In very ill, and particularly in elderly, patients the tube could cause an accumulation of mucus and interference with swallowing, so that aspiration into the trachea might result.

Dr. K. N. V. Palmer asked how often aspirin was incriminated in patients with acute gastric erosions, and whether achlorhydria was a common finding in patients in whom haematemesis appeared to be due to aspirin.

Dr. Oliver Fitzgerald commented on the use of the term achlorhydria in the presence of a pH as low as 4. He pointed out that such gastric H-ion concentrations were usually associated with the secretion of hydrochloric acid in small amounts. This small amount of acid was of course soon neutralized, but could potentially lead to mucosal damage before neutralization.

Dr. E. N. Rowlands asked whether the secretory studies were repeated after an interval of weeks or months following the haematemesis, and if so, what percentage of those who were achlorhydric showed a return of acid secretion.

Dr. D. A. K. Black asked whether any evidence of oesophagitis had been found in those few patients who came to autopsy after intubation.

In reply, Dr. Chandler said that consideration had been given to gastric biopsy, but it had not been done because of the hazards of a potentially aggravating procedure in a patient with haematemesis. The emptying rate of the stomach had not been measured, since as a group the patients were unsuitable for methods of investigation such as serial test-meal analyses. It had not been found possible to advance the tube into the duodenum to distinguish between duodenal and gastric bleeding. He had not been impressed by any greater liability on the part of these patients to develop respiratory complications. The procedure was designed to provide an early aetiological diagnosis, and the majority of the patients with continued bleeding were submitted to surgery at an early stage. A history of previous aspirin medication was obtained from nine patients in the acute group, of whom five were achlorhydric. The term achlorhydria had been used in a conventional sense, and the presence of hydrochloric acid at the pH at which Töpfer's reagent changed colour (pH 3–4) was not disputed. No evidence of oesophagitis was found in the few patients who had come to autopsy.

6. Dr. K. B. Taylor (introduced) and Professor L. J. Witts described the *Effects of Chronic Iron Deficiency in the Rat*. Using a semi-synthetic diet containing less than one part of iron per million, and specially designed 'perspex' cages, it had been possible to produce and maintain for over one year animals with haemoglobin concentrations below 50 per cent. of normal and serum-iron concentrations less than 25 per cent. of normal. Mortality of the animals was high. Many deficient animals showed impaired production of gastric free hydrochloric acid, impaired absorption of vitamin B_{12}, and impaired secretion of Castle's intrinsic factor, measured by testing the ability of their gastric juice to restore the absorption of vitamin B_{12} in rats subjected to gastrectomy. The secretion of pepsin was unimpaired. Two iron-deficient animals, in which the deficiency was corrected, recovered the ability to secrete free acid. The gastric mucosa of some test animals showed

varying degrees of atrophy; in one rat this was severe and generalized, presenting an appearance similar to that seen in pernicious anaemia.

DR. C. F. HAWKINS asked whether there were any histological changes in the small intestines of these iron-deficient rats.

DR. P. FOURMAN asked whether the anaemic rats had atrophic changes in the tongue or the nails.

DR. SHEILA CALLENDER said that, in patients with iron-deficiency anaemia in whom vitamin-B_{12} absorption was impaired, no improvement in absorption of vitamin B_{12} had been observed after recovery from the iron deficiency. In particular one woman, who first suffered from hypochromic anaemia in 1952, had been in perfect health since receiving iron therapy alone. Repeated observations of her serum vitamin-B_{12} level and absorption of ^{60}Co–vitamin B_{12} had, however, shown that both remained persistently low.

DR. J. M. LEDINGHAM asked whether the chronic anaemia, rather than iron deficiency, could be the cause of the decreased production of hydrochloric acid and intrinsic factor, and suggested that a trial might be undertaken in which a comparable degree of chronic anaemia was induced by repeated bleedings with adequate iron replacement.

DR. OLIVER FITZGERALD said that refection was a normal phenomenon in the rat. The elimination of this factor by the use of special cages, and the feeding of an iron-deficient diet, might lead to more complex changes, such as ascorbic-acid deficiency, than would be produced by the diet alone.

In reply, DR. TAYLOR said that no changes had been noted in the mucosa of the small intestine, in the claws, or in the tongue. In his experience the normal rat's tongue often showed areas of depapillation and flattening. The cause of death in the test animals was not obvious. It was not due to cardiac failure: the hearts of the test animals were of normal weight and size, unlike those reported in earlier work by Schmidt on rats fed on rice diets, where a vitamin-B-complex deficiency might also have been present. Preliminary studies had shown that the cytochrome-C content of heart muscle was not lower in the test animals. He knew of no reliable evidence of abnormally low vitamin-B_{12} absorption in patients with chronic iron deficiency being corrected when the iron deficiency was made good. The refection of faeces played no part in the vitamin-C economy of the rat, which synthesizes its vitamin C and does not rely on intestinal flora.

7. DR. R. O. K. SCHADE (introduced by DR. H. G. MILLER) described *The Cytodiagnosis and Relationship of Chronic Atrophic Gastritis and Carcinoma*. He pointed out that the cytology seen in cases of chronic gastritis—plasma cells, erythrocytes, polymorphs, and epithelial cells from areas of intestinal metaplasia—was always found in cases of carcinoma, particularly in the early stages. The clinical picture, which in many instances is identical in carcinoma and chronic gastritis, was further evidence for a relationship between these two conditions. In a series of histological preparations he showed how chronic gastritis with intestinal metaplasia might change into 'surface carcinoma', which is the origin of the commonly observed gastric carcinoma. Lastly he referred to evidence that gastric carcinoma never arises in a normal mucosa, and to the increased incidence of carcinoma in patients with pernicious anaemia who also had atrophic gastritis.

After the morning session the President paid a warm tribute to PROFESSOR SIR L. S. P. DAVIDSON for the excellence of the Association's meeting in Edinburgh in 1957, and thanked Sir Stanley for having been such an excellent President.

After lunch, kindly provided by the Board of Governors in the Nurses' Home at the General Infirmary, the afternoon was devoted to a large number of clinical and scientific demonstrations most ably organized by members of the clinical and preclinical staff of the Leeds Medical School.

Annual Dinner

The Annual Dinner was held in University House, the Leeds members kindly providing sherry beforehand. The President, Dr. J. R. H. Towers, was in the Chair. The toast of the Association was proposed by the President, and that of the guests by Professor R. E. Tunbridge. The Vice-Chancellor of the University, Sir Charles Morris, replied.

Saturday Morning

8. DR. B. H. MCCRACKEN and DR. F. M. PARSONS (introduced by PROFESSOR R. E. TUNBRIDGE) outlined the treatment of acute renal failure with glucose and water, and

described *Anabolic Steroid Therapy in Acute Renal Failure*, because the prognosis largely depended upon the extent to which protein katabolism could be reduced. They used as the index of protein katabolism the amount of urea produced per day, and this was determined by adding the daily excretion of urea, if any, to the daily accumulation in body fluids. Urea production figures were presented for five patients with acute renal failure due to complications of pregnancy, five patients with acute renal failure from other causes, and four normal subjects on a dietary régime similar to that used in acute renal failure. Each subject was treated with 'nilevar' (17-ethyl-19-nortestosterone) 80 mg. by mouth daily for five days after a control period of four days. The pregnancy patients showed a dramatic reduction in urea production when given nilevar, the mean on treatment days being only 30 per cent. of the control value, in contrast to the other patients and normal subjects, who showed only small inconstant reductions. It was concluded that nilevar reduced protein katabolism in acute renal failure following pregnancy complications to an extent that might eliminate the need for artificial kidney treatment in this group of patients.

Dr. J. D. N. Nabarro pointed out that nortestosterone had a progestational action, and wondered whether the apparent benefit in the obstetric cases was due to inhibition of involution of the uterus. Dr. McCracken agreed that this might be the explanation.

9. Dr. H. E. de Wardener and Dr. E. D. Barlow (introduced) had studied *Compulsive Water Drinking* in seven women and two men, whose ages ranged from 48 to 59 except for one patient aged 24. Both age and sex distribution agreed with the only 10 cases mentioned in the literature, and differed from diabetes insipidus, which usually starts in childhood and is equally common in either sex. Eight of the nine compulsive water drinkers had histories of previous psychological disturbances ranging from delusions, depression, and agitation to frank hysterical behaviour; only one patient appeared normal. The consumption of water fluctuated irregularly from hour to hour and from day to day; in some patients there were remissions and relapses lasting several months or longer. After investigation a distinction was made from 14 cases of diabetes insipidus by the results obtained from the plasma osmolarity, the effect of aqueous vasopressin and of vasopressin tannate in oil, and the effects of fluid deprivation. In compulsive water drinking plasma osmolarity was lower, whereas in diabetes insipidus it was higher, than normal. In both conditions the ability to concentrate the urine after aqueous vasopressin was variable; the greatest impairment occurred in compulsive water drinkers. Vasopressin tannate in oil made compulsive water drinkers ill, but made patients with diabetes insipidus better. The urinary changes following fluid deprivation usually distinguished the two conditions, but only when they were interpreted in conjunction with the effects obtained with intravenous aqueous vasopressin. In normal subjects the urine concentration after fluid deprivation was greater than after a saturating dose of vasopressin. Dr. de Wardener therefore assumed that if, in a patient with polydipsia and polyuria, the urine concentration after fluid deprivation was greater than after a saturating dose of vasopressin, the ability to secrete antidiuretic hormone (ADH) was normal, whereas, if the urine concentration after fluid deprivation was less than after the vasopressin, the ability to secrete ADH was impaired. Accordingly, six patients with compulsive water drinking were considered to be able to secrete ADH normally, but in two the ability to secrete ADH was impaired. After a remission of the compulsive water drinking, however, these two patients had a normal response to fluid deprivation. All the patients with diabetes insipidus had an impaired ability to secrete ADH. In four patients with compulsive water drinking the fluid intake returned to normal after electro-convulsive therapy or a period of continuous narcosis; the improvement in three was transient, but in the fourth it had lasted two years.

Dr. M. D. Milne asked whether there would have been as clear-cut a separation of patients if serum-sodium estimation had been used, rather than the more difficult and less generally available method of serum osmolarity.

Dr. C. L. Cope suggested that the diagnoses of diabetes insipidus and of compulsive water drinking need not be mutually exclusive. He had studied one patient in whom the conclusion had to be drawn that a moderate degree of diabetes insipidus was associated with a considerable degree of compulsive water drinking. Diabetes insipidus imposed a severe mental stress, which apparently induced a psychogenic overlay.

Professor J. R. Squire asked what steps had been taken to exclude chronic nephritis in the broadest sense as a cause of the polyuria.

Dr. D. A. K. Black asked whether the hypertonic saline test of Hickey and Hare was of any value in distinguishing between diabetes insipidus and functional polydipsia.

DR. J. MARSHALL asked whether compulsive drinking was episodic in any of the cases, and whether it was associated with compulsive eating, hypersomnia, or hypnagogic hallucinations.

DR. P. FOURMAN asked whether there was any way of distinguishing those cases of primary polydipsia in which excessive drinking was the result of a lesion of the thirst centre in the hypothalamus (Kourilsky, David, Sicard, and Galey (1942) *Rev. neurol.* **74**, 264).

DR. S. W. STANBURY suggested that the compulsive drinker's inability to concentrate the urine after vasopressin administration must be regarded as an acquired functional disorder of the renal tubules. He asked whether other functions of the renal tubules had been specifically tested, and whether studies had been made of the pattern of recovery of the capacity to concentrate urine.

In reply, DR. DE WARDENER said that the plasma-sodium concentration had not been measured in all cases. He agreed that emotional inhibition of ADH secretion might be the initial cause of thirst and polyuria, but he thought the impairment of ADH secretion observed in two of his patients was a secondary phenomenon, otherwise vasopressin tannate would not have made them feel worse. He assured Professor Squire that none of the compulsive water drinkers had evidence of renal disease. He wished he had performed a hypertonic saline test more often and compared the results with those obtained from fluid deprivation. In the two patients with compulsive water drinking in whom this test was performed there was a simultaneous osmotic salt diuresis, which made it difficult to gauge whether ADH hormone secretion had occurred. The crux of the problem, when differentiating compulsive water drinking from diabetes insipidus, was not to be satisfied with only one test if it suggested that the ability to secrete ADH was abnormal; conversely, if any one test suggested that the ability to secrete ADH was normal, the diagnosis of diabetes insipidus was unlikely. Dr. de Wardener had never seen a case of polydipsia and polyuria caused by a lesion of the thirst centre. He supposed it might be possible to differentiate the condition from compulsive water drinking by an absence of fluctuations in polyuria. In reply to Dr. Stanbury, he said he had some evidence that drinking large quantities of water would itself impair the kidneys' ability to concentrate. If normal subjects drank about 10 litres of water a day for 11 days there developed an ability to concentrate from about 900 to 800 m-osmole/l. to approximately 400 m-osmole/l. Nevertheless other factors must also be involved, because the patient with compulsive water drinking who drank up to 20 litres a day was able to concentrate her urine normally.

10. DR. J. F. GOODWIN, and DRS. W. B. BURACK and J. PRYCE (introduced), described a *Reversible Nephrotic Syndrome in Heart Failure*. The typical picture, with gross proteinuria, massive oedema, hypoalbuminaemia, and hypercholestrolaemia, had developed during treatment for chronic congestive heart failure with mercurial diuretics. Complete recovery occurred in three patients on continued treatment which included mercurial diuretics; the fourth did not improve. Recognized causes of the nephrotic syndrome were excluded in life by renal biopsy and inferior vena caval angiography in two cases, and at autopsy in the other two. Urinary mercury estimations were carried out in two cases, and the excretion was increased in one. Dr. Goodwin concluded that mercurial diuretics did not predispose to, or cause, the syndrome, which was probably related to renal circulatory changes resulting from heart failure.

PROFESSOR G. A. SMART, DR. R. L. RICHARDS, and DR. E. G. WADE described similar cases. Dr. Richards's patient had received mercurial diuretics for a prolonged period after a myocardial infarction. None of the usual causes of the nephrotic syndrome was present. This patient differed from those described by Dr. Goodwin in that after the cardiac infarct he had not had congestive failure, and it was thought more likely that the nephrotic syndrome was due to prolonged administration of mersalyl. In the two patients described by Dr. E. G. Wade the syndrome persisted until death from heart failure, and was unaffected by withdrawal of mercurial diuretics or the administration of potassium supplement. Detailed post-mortem studies of the kidneys failed to reveal any abnormality.

In reply to DR. W. T. COOKE, DR. GOODWIN said the serum-electrolyte values were normal or consistent with heart failure in all his cases. He assured Dr. de Wardener that the histology of the renal tubules was normal.

11. DR. A. A. F. PEEL, using a method devised by Hartert for recording the physical process of blood coagulation, discussed *Thrombo-elastography in the Investigation of Thrombosis and in the Control of Anticoagulant Treatment*. He described the variations in the

thrombo-elastogram resulting from the addition of heparin *in vitro*, from heparin therapy, and from depression of the prothrombin level by dindevan. Thrombo-elastographic findings suggested that some patients produce an autogenous anticoagulant with a heparin-like action in response to thrombosis. The production of this anticoagulant factor during the course of dindevan therapy was a possible explanation of the occasional occurrence of haemorrhage at the time when the prothrombin level was considered satisfactory.

DR. E. G. WADE asked whether Dr. Peel had used the thrombo-elastogram to control anticoagulant therapy, and how it compared with the orthodox prothrombin index method. Was there evidence that changes in the thrombo-elastogram preceded embolic episodes?

DR. T. E. GUMPERT pointed out that most fatal myocardial infarcts proved at post-mortem examination to be due to calcification of the coronary arteries rather than to coronary atheroma with intimal plaques. In these calcified arteries the lumen would barely admit the passage of a pin, and there was no demonstrable clot. He felt that the significance of thrombosis itself, as opposed to ischaemia, was probably overemphasized.

DR. W. WALKER said that, in view of the great increase in long-term anticoagulant therapy in out-patients that was likely in the near future, any procedure that improved our understanding of the mode of action of coumarin drugs, or made the control of treatment simpler and safer, was more than welcome. He wondered, however, whether the thrombo-elastogram gave any information not obtainable by simpler and more specific procedures; and asked whether the findings (1) correlated with any particular pattern or patterns of coagulation defect; or (2) gave any surer indication of therapeutic effectiveness or risk of haemorrhage.

In reply, DR. PEEL said he still used the prothrombin time as a routine for the control of anticoagulant treatment, with thrombo-elastograms in addition whenever possible. If a haemorrhagic tendency were suggested by either method the dose of anticoagulant was reduced. He had not so far had an opportunity to carry out thrombo-elastograms shortly before a cardiac infarct.

12. DR. E. W. SKIPPER described five cases of *Pulseless Disease* (*Takayasu's Disease*), in which an obliterative process occurring at or just above the origins of the arterial branches of the aortic arch was responsible for absence of palpable arterial pulsation in the upper extremities and usually in the head and neck. Although Davey described, in 1839, a case due to syphilitic aortitis with aneurysm of the arch, the condition had excited little interest in this country until recent years. The principal symptoms were recurrent syncopal attacks with transient dimness of vision sometimes leading to blindness, major cerebral episodes such as infarction, and intermittent claudication of the arms. In all five cases arterial pulsation was absent in the upper limbs, and the blood-pressure could not be recorded in them; in three carotid pulsation was also absent, and in two of these 'fragmentation' of the retinal circulation was observed, with eventual cataract formation. In three patients a collateral circulation could be felt over the back of the chest, and in three hypertension was present in the legs. Autopsy in a woman aged 44 showed the stenosis of the vessels to be due to a non-specific arteritis with lymphatic infiltration, chiefly of the adventitia. The arch of the aorta showed similar histological features, aneurysmal dilatation of the ascending portion, and an area of coarctation, insufficient to cause obstruction, immediately distal to the great vessels. The innominate and left common carotid arteries arose from a common root. In the other fatal case, a man aged 47, the occlusion was due to thrombosis within severely atheromatous vessels. In this man no pulsation was felt in the lower limbs below the groins. In two patients no symptoms could be ascribed to the pulseless disease, the condition being found during routine examination in a mental hospital. The cause of pulseless disease may be syphilitic arteritis, atheroma, or non-specific arteritis, the latter appearing to be the most common especially in young female subjects.

DR. R. W. D. TURNER referred to a woman aged 40 who had been under observation for 10 years with aortic incompetence, absent pulsation in the left common carotid artery, and delay in the left radial pulse. Six years ago she developed severe anginal pectoris, and since that time had been known to have a persistently raised sedimentation rate. Recently she had developed dimness of vision from retinal artery occlusion with typical peripapillary haemorrhages, as described by Takayasu. He asked whether the association of aortic incompetence with the aortic arch syndrome had been noted before, whether coronary arteritis had been described, and whether there was any evidence that treatment with steroids or long-term anticoagulant drugs might be effective.

DR. E. G. L. BYWATERS asked whether the Wassermann reaction was negative.

DR. D. A. D. MONTGOMERY said that the response to cortisone had been gratifying in one case. The patient, a 48-year-old woman, had severe pain in her arms at rest, and claudication of arms and legs, with a raised sedimentation rate (62 mm. in one hour) and an abnormal electrophoretic pattern. After prednisolone treatment pain was considerably improved, while the sedimentation rate and electrophoresis returned to normal. He considered that steroids might prevent the dangerous cerebral complications which commonly occur in the active phase of the disease, and they were well worth a trial in cases resulting from inflammatory arterial disease.

DR. J. L. LIVINGSTONE quoted the case of a man aged 35 years, with claudication of the arms and pain radiating into the left neck. He was investigated by Dr. Paul Wood and referred to Professor Robb, who performed a bilateral subclavian arterial graft; the vessels became occluded after a few months.

PROFESSOR A. W. WOODRUFF said the condition described by Dr. Skipper bore certain resemblances to endomyocardial fibrosis, and asked whether there had been any fibrosis beneath the endocardium.

DR. R. S. BRUCE PEARSON said that in a case of this type occurring in a man of 29, who had obliteration of the brachial and carotid pulses due to atheromatous changes and subsequent thrombosis, both renal arteries were also obstructed where they arose from the aorta. It was considered that a naturally occurring Goldblatt experiment had taken place, and that the hypertension was related to the reduced blood-supply to the kidneys (*J. Path. Bact.* (1946) 58, 564).

In reply, DR. SKIPPER said that corticosteroids were indicated when the sedimentation rate was raised, and that nylon by-pass grafts were also promising. The Wassermann reaction had been negative in his cases, and there had been no evidence of subendothelial fibrosis.

13. PROFESSOR A. A. MONCRIEFF spoke on *Biochemistry and Mental Defect*. Recent metabolic studies had offered important information as to the possibility of preventing certain types of mental defect by early detection and treatment. Historically the cretin could be regarded as an example of a metabolic disorder causing mental deficiency, and on the chemical side lead poisoning could certainly wreck the brain. The lipoid storage disorders had long been known to be associated with mental defect. More recently it had been shown that phenylketonuria could be treated, and this also applied to galactosuria and to nephrogenic diabetes insipidus. Sucrosuria was also associated with mental defect, but did not apparently yield to treatment. The lipoidoses represented an important hereditary group, with mental defect and abnormal lipoids in the brain. As a result of chromatography aminoacidurias of various types were being reported frequently. Nephrogenic diabetes insipidus was probably transmitted as a sex-linked recessive, and adequate water intake could transform an ament into a normal child.

In reply to DR. J. M. STOWERS, PROFESSOR MONCRIEFF said that sucrosuria depended upon the ingestion of sucrose.

14. PROFESSOR E. G. L. BYWATERS, and DRS. M. E. CARTER and F. SCOTT (introduced), reviewed the problem of whether children with chronic polyarthritis suffered from *Still's Disease or Rheumatoid Arthritis*, and described the differences or similarities between 142 juvenile patients with polyarthritis and 140 adult patients with rheumatoid arthritis, the two groups being similar in duration of follow-up, diagnostic criteria, sex incidence, and duration from onset when first seen. The clinical features were similar in both groups, but occurred in different proportions. Patients with juvenile rheumatoid arthritis showed a greater incidence of rashes, fever, and splenomegaly, and less nodules than adults. Each of these features, however, and the results of the Rose–Waaler test, changed progressively with age. The difference in the histology of nodules was illustrated, showing the closer resemblance of Still's nodules to rheumatic fever than to adult rheumatoid nodules, but this too could be attributable to differences in age. The pathology of joints was the same if allowance was made for the greater thickness of cartilage in young children. Dr. Carter concluded that clinically there was not enough difference between the juvenile and adult polyarthritis to justify calling them separate diseases, and that such differences as there were could be attributed to differences in age.

PROFESSOR J. H. KELLGREN asked how many of the children with polyarthritis were excluded because they were considered to be suffering from other forms of arthritis. Was the proportion the same as in adults, or were all the children with chronic polyarthritis

accepted as rheumatoid? In reply, DR. CARTER said the juvenile and adult groups were closely comparable.

15. DRS. J. SHARP, J. BALL, and J. S. LAWRENCE (introduced by PROFESSOR J. H. KELLGREN) described *Some Neurological and Other Aspects of Instability of the Cervical Spine in Rheumatoid Arthritis and Ankylosing Spondylitis*. Dr. Sharp gave an account of 29 patients with rheumatoid arthritis and 19 with ankylosing spondylitis who had developed vertebral displacements and abnormal mobility in the cervical spine; 15 of them had sustained damage to the cervical cord. In 45 patients, 12 of whom had cord damage, the major or only displacement was at the atlanto-axial level. In three patients the diagnosis had been made only at autopsy. Six had presented neurological symptoms, 20 local pain, and four occipito-cervical deformity from atlanto-axial displacements. In 15 patients without neurological changes, of whom 13 had rheumatoid arthritis, an atlanto-axial displacement had first been diagnosed on a routine radiograph of the cervical spine. Accounts of several patients were given to illustrate the difficulties in diagnosis and the variation in severity of the neurological changes, which ranged from complete tetraplegia with anaesthesia up to the lower jaw to mild pyramidal and sensory disturbances. The results of treatment of patients with neurological complications by skull traction and occipito-cervical fusion had been very satisfactory, and only two had had serious neurological residua. Five patients without cord damage had required operation for intractable local pain or deformity from atlanto-axial displacements, but the remainder had been satisfactorily managed in supporting collars. Pathologically, rheumatoid arthritis of the cervical spine was characterized by loss of stability from erosion of intervertebral disks and vertebral bodies, by granulation tissue arising in the neuro-central joints, and by a similar destructive process in the apophyseal joints. The resulting radiological changes were narrowing of multiple disks, especially the upper two, with erosion rather than proliferation of the vertebral bodies, vertebral subluxations, often multiple, and erosion of apophyseal joints. These changes were recognized with reasonable reproducibility in 6 per cent. of male and 7 per cent. of female patients in 428 X-rays of a random sample of the general population aged 55 to 64. They showed a highly significant association with positive sheep-cell tests, and to a lesser degree with radiological changes typical of rheumatoid arthritis in the hands or feet, but only a poor association with rheumatoid arthritis as diagnosed clinically. There was evidence that this may be because rheumatoid arthritis may occur in a form in which the spine is mainly or exclusively involved, in which case a clinical diagnosis of rheumatoid arthritis may not be considered.

PROFESSOR A. V. NEALE said he thought that Dr. Carter had made it fairly clear that Still's disease and rheumatoid arthritis were essentially the same pathological process, and in children acute or subacute periarthritis in the cervical region could cause partial or asymmetrical dislocation. In one patient there had been evidence of unilateral cord compression which was completely relieved by reduction of the abnormal vertebral position.

PROFESSOR E. G. L. BYWATERS said he had not seen neurological changes in children with Still's disease.

DR. J. MARSHALL asked what was the mechanism of the cord lesion. The fact that one patient recovered after many months was very much against a vascular mechanism, as vascular lesions of the spinal cord rarely showed any appreciable recovery.

DR. J. B. RENNIE asked whether any of the patients had been treated with steroid hormones.

In reply, DR. SHARP stated that he had no information as to the prevalence of atlanto-axial displacements in juvenile rheumatoid arthritis. It might be expected that in childhood there would be an increased tendency to vertebral displacements from the increased mechanical stresses on the unfused spinal segments. The study of three spinal cords was not yet complete, but in one, from a patient with gross atlanto-axial dislocation, the appearances were compatible with direct mechanical injury by the odontoid process. In the others there were scattered focal lesions, not only at the level of the vertebral displacements, but also above and below this level. They were not confined to the territory of the anterior spinal artery. It was not at present clear how these lesions had been produced.

MASSIBA

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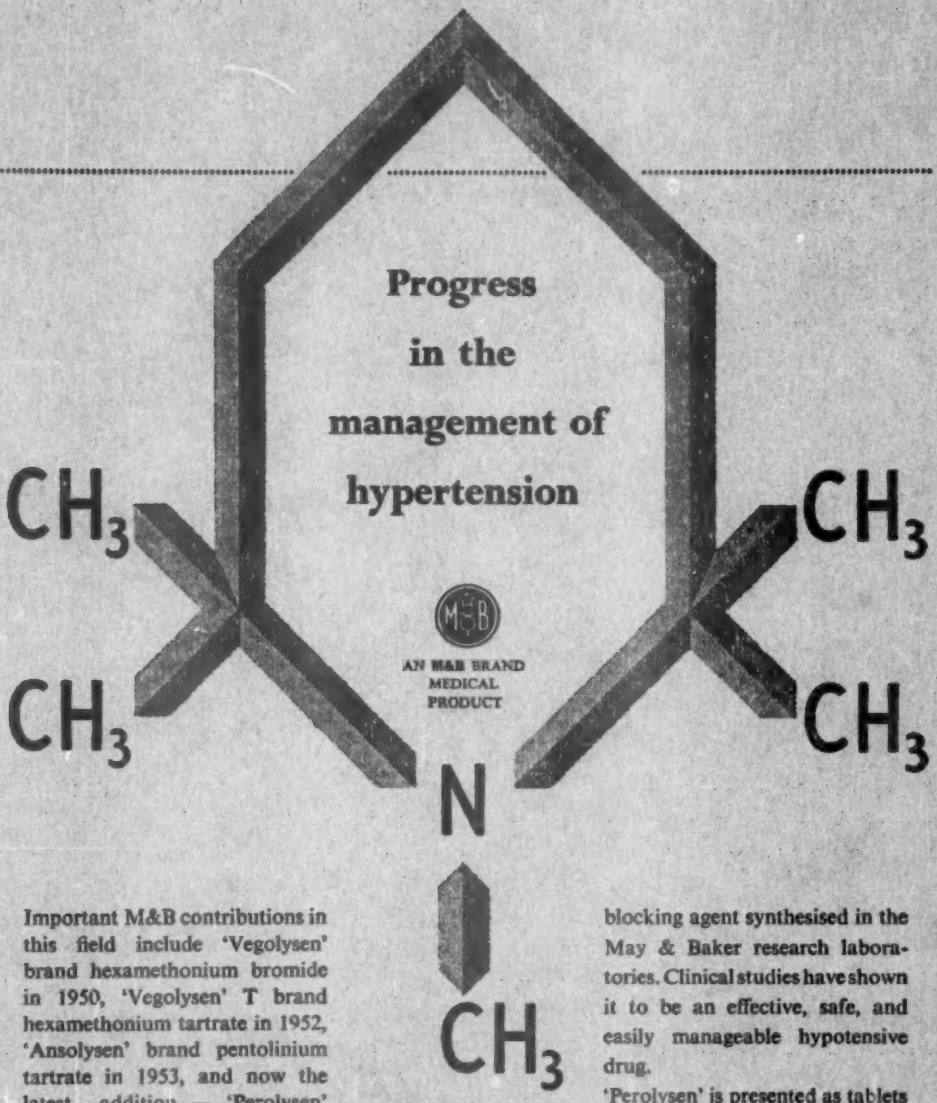

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